

Dr. Graham - for your info.



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Administration~~

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Screwworm Research
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October 8, 1981

SUBJECT: Transmittal of Report on Screwworm Genetics

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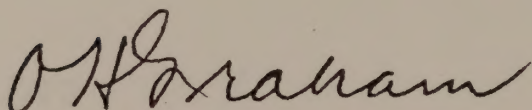
A copy of a report, "Genetic Studies of Screwworm Populations in Chiapas, Mexico (April-August 1981)", prepared by D. O. McInnis and associates is forwarded as an enclosure to this memorandum. This is primarily a report of a comparison of a narrow genetic base strain with a broad genetic base strain that was made on the Pacific Coast of Chiapas in Cooperation with a University of Texas research group. In part, the UT group was funded by USDA, Cooperative Agreement 58-7B30-9-120.

By mutual agreement, the insects collected during the test were divided equally between the UT and USDA groups. Dr. Richardson and associates have analyzed part of their material and are currently preparing their report; we understand it will differ quite a bit from Dr. McInnis's report.

You will note that genetic analyses completed by Dr. McInnis do not support the UT contention that the population in southern Chiapas contains 7 gamodemes (reproductively isolated types). At the bottom of p. 20, McInnis et al states, "----no recognizable pattern emerged to suggest the existence of two or more discrete (or diagnostic) karyotype sets representing distinct, yet coexisting (sympatric) screwworm populations (gamodemes)." Dr. McInnis has found extensive heterogeneity within egg mass groups (i.e., sibships) and heterozygosity between recognized karyotypes and has concluded (see p. 34), "Native screwworm flies in the Arriaga area demonstrated polymorphic chromosome variation characteristic of a single gamodeme."

In a letter dated Sept. 23 Dr. Richardson notes that his group saw "an asexual screwworm case" in the material from Chiapas and suggest that a parthenogenetic type of screwworm might be selected out by the release of sterile males in areas of southern Mexico. (The implication is that parthenogenetic females would replace all other screwworms and thereby defeat the eradication program). Dr. McInnis (see p. 26) suggests that the two triploid individuals found by the UT group were more likely the result of "a freak developmental mutation" than parthenogenesis. There is no evidence for asexual reproduction in screwworms other than the two triploid individuals.

You will also note that McInnis et al feels that in the Arriaga area mating preferences may influence mating in nature to produce a form of assortative mating. Such mating preferences are not mating barriers and are not evidence that the present sterile male release program is doomed to fail (in southern Mexico) as the UT group have contended. Nor is it evidence that a strain tailor-made for use in a particular area would be 4 to 10X more efficient than the strains which have been used in the program. However, Dr. McInnis feels that we should take advantage of apparent mating preferences anywhere they actually exist by matching chromosome patterns of the factory strain with those of the target population.



O. H. GRAHAM

Location/Project Leader

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Enclosure

V-81 Strain Evaluation (1981)

Sterility data

TEST AREA (9 pens)

<u>Week</u>	# Sterile Masses	# FERTILE MASSES	% STERILITY	Release Dates	MISC.
8/10-8/16	1	72	1.4	—	PRE-RELEASE
8/17-8/23	1	45	2.2	—	"
1. 8/24-8/30	5	42	10.6	8/24, 8/27, 8/28	No release on 8/25 Heavy rain until 8/29
2. 8/31-9/6	19	109 119	14.8 13.4	[Scheduled] 8/31, 9/1, 9/3, 9/4	Delays in releases due to low emergence
3. 9/7-9/13	34	103	25.0	9/8, 9/9, 9/11, 9/12	
4. 9/14-9/20	10	41	19.6		
5. 9/21-9/27	13	67	16.3		
6. 9/28-10/4 (6 days)	13	63	17.1		
7. 10/5-10/11	7	51	12.1		
8. 10/12-10/18	13	63 50	20.6		
9. 10/19-10/25	34	69	33.0		
10. 10/26-11/1 (last release on 10/31)	28	46	37.8		
11. 11/2-11/7 (6 days)	26	43	37.7		

From McInnis report of
10/05/81

From analysis of 23 pre-release egg mass groups:

(1). In general the same conclusions were drawn from computer analysis and visual analysis but computer analysis revealed statistically significant variation between individuals within an (egg mass) group.

(2) a significant level of within-group chromosome heterogeneity was found.

(3) on the basis of ~~exa~~ samples analyzed to date, no recognizable pattern emerged to suggest the existence of two or more discrete (or diagnostic) karyotypes sets representing distinct sympatric populations (gamodemes).

(4) For each of the 25 computer analyzed groups, including RGM-6 and V-81, at best only a poor fit could be made to any of the nine gamodeme types currently recognized by the UT group. In addition, ~~each~~ ~~of~~ data from several (egg mass) groups could each be fitted to several UT-recognized gamodemes.

Under the heading "Polymorphic chromosomes", a total of 27 variants were seen. The variations were probably produced (see p. 25) following a series of chromosomal duplications, deletions, and possibly translocations or inversions in a couple of original types. Most of the variants have previously been seen in one or more of the northern

Chemistry

Support of SWASS manufacture
Modification of swamplure -
Larval and adult nutrition

states of Mexico. A variation seen only once, III-2, contained two triploid individuals (found by the UT group). The UT researchers have concluded that these triploid individuals are evidence that diploid females are being produced parthenogenetically. McInnis suggests that a freak developmental mutation producing diploid eggs is a more likely cause of the triploidy than parthenogenesis.

GENETICS STUDIES OF SCREWORM POPULATIONS
IN CHIAPAS, MEXICO
(APRIL - AUGUST, 1981)

by

D. MCINNIS, C. J. WHITTEN, J. SPENCER, J. MACKLEY, R. PETERSON

(prepared Oct. 5, 1981)

USDA, ARS, ~~ARS~~
SCREWORM RESEARCH

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INTRODUCTION

As the screwworm eradication program advances further south into Mexico the sterile released flies will come into contact with wider environmental diversity and year-round native populations. Therefore, it has become increasingly important to characterize the genetics of target screwworm populations in order to maximize the mating compatibility of released strains. The genetics of mass-produced insects has been examined (Bush and Neck, 1976; Kaufman and Wasserman, 1957; Whitten, 1980), but knowledge of the extent and pattern of variation indicating assortative mating among native flies would be of particular concern since the sterile released flies may also demonstrate differential mating abilities with genetically distinct native flies. Herein is described a large scale field test designed to elucidate the pattern of chromosome variation among native screwworms of southern Mexico, as well as to assess the extent, if any, of assortative mating by two genetically distinct sterile released strains.

MATERIALS AND METHODS

Objectives of the field test:

The primary objectives of the field test near Arriaga in Chiapas, Mexico were to determine (1) the extent, if any, of sexual isolation among the types of screwworms in the area, and (2) the comparative effects of releasing a narrow genetic base (single egg mass) strain and a broader genetic base (multiple egg mass) strain.

Additional secondary objectives of interest included a preliminary investigation of mass-rearing characteristics of narrow and broad based release strains, and recommendations for the future direction and operation of the eradication program.

The Test Area:

The test area encompassed a narrow strip of coastal plain in the Mexican state of Chiapas extending from near Arriaga eastward to near Tapachula (see Figure 1 map). Three test zones were established within the entire area (ca. 4800 km²). Zone A had initial dimensions of 76 x 18 kilometers (1368 km²) near Arriaga and was treated with sterile flies of the narrow genetic base strain (designated by Univ. of Texas researchers). On July 5th the dimensions of Zone A were reduced to 55 x 18 kilometers (990 km²). Zone B, initially 1160 km² (58 x 20 kilometers) and later 2220 km² (111 x 20 kilometers), lay in between Zones A and C. Zone B received no released sterile flies. Zone C, near Tapachula, was originally 2160 km² (72 x 30 kilometers) but was reduced to 1200 km² (40 x 30 kilometers) simultaneously with the changes made to Zones A and B. The boundary alterations were made in an effort to reduce the observed sterility in the critical 'control' area, Zone B. The release areas

were reduced so as to increase the distance separating sheep pens in Zone B from the drop zones.

Sampling Procedures:

Sentinel sheep pens and wind-oriented, Swormlure baited traps were used to collect egg masses and adult insects in the three zones. Five sheep pens and ten traps were set in place and operating by April 25th in each zone (see Figure 1 for pen locations). Each sentinel pen contained two sheep which had wounds infested with larvae as attractants for native females. Egg masses were collected from each pen at least once, and usually twice, daily. These were transported to the research facility in Tuxtla-Gutierrez every day. Egg masses were labelled and hatched larvae were reared on standard meat media. Third instar larval samples were collected and frozen in liquid nitrogen for later genetic analysis. Duplicate samples were taken for nearly all cultures and transported to both the Univ. of Texas and SEA/AR labs in Austin and Mission, TX., respectively.

Adult flies were collected in the wind-oriented traps or off the sentinel sheep. These insects were labelled, stored in liquid nitrogen, and returned to the Mission lab for later dissection and genetic analysis. Since the latter did not bear directly upon the objectives at hand, a discussion of the results from adult analyses will appear in a separate, subsequent report.

Both the Univ. of Texas and Mission researchers utilized the same genetic technique for chromosome preparation developed by John Ellison (Univ. of TX). This involved dissecting out and squashing the larval brain, then preparing a slide after several chemical treatments including staining with a fluorescent dye. Mitotically dividing cells with visible chromosomes were observed with

UV light microscopy, photographs were taken, and print enlargements were made. The chromosomes were then measured and the numerical data subjected to rigorous computer-assisted analysis. After the analysis was completed on a sample of pre-release egg mass groups, it was determined that larvae could be genetically scored at the microscope and print enlargement stages.

The first author examined a minimum of five cells containing all the genetic information per individual larva, and a minimum of five individuals per egg mass sibship, including at least two of each sex. Based upon the chromosome constitution of sibling larvae, the genetic configuration of the two native parents could be determined. It is from the latter information about a collection of native insects that an inference can be drawn regarding assortative mating. If the sampling per egg mass could not reach the minimum standards described above, then only partial information was inferred as to the constitution of the native parents. It is the opinion of this first author that a large part of disagreements in the interpretation of results arises directly from differences in the amount of within group sampling deemed adequate (# cells/individual and # individuals/sibship).

Pre-release egg mass sampling continued in all three zones for a period of ca. six weeks (4/25-6/4). During this time the narrow-based, single egg mass strain (RGM-6) and the broader-based, multiple egg mass strain (V-81) were being expanded and prepared for release by the Methods and Development section at the production facility in Tuxtla-Gutierrez. The RGM-6 strain originated from a collection made in the Arriaga area within the test area during August, 1980. The V-81 strain originated from sixteen separate egg mass lines collected from sheep hosts near Veracruz, Mexico during June and July, 1980. The six-

teen lines were systematically combined into a single strain through reciprocal forced crosses. The V-81 strain was thus prepared in the manner of most previous factory strains. The former was itself earmarked for mass production in October, 1981 pending successful evaluation at the conclusion of the assortative mating test. A discussion of the field evaluation of the V-81 strain will be included in the follow-up report mentioned above. At the time of the final combination creating the V-81 strain, ten or eleven generations had passed from the time of collection, depending upon the particular egg mass component. By that time the RGM-6 strain was in its ninth generation (F 9). Larval samples for genetic analysis were periodically taken for both strains during the pre-release and post-release periods. Certain quality control rearing data were also collected for both strains. Larval sampling continued, of course, from egg masses collected in the test zones throughout the post-release period.

Sterile fly releases:

Releases of the V-81 strain in Zone C began on June 5th and of the RGM-6 in Zone A on June 6th. Two releases per week for each strain were planned. Flies were aeri ally released in standard small boxes (400 flies capacity) at customary plane speed (135 mph) and altitude (ca. 500 meters), perpendicular to the coast. Flies were dispersed along two mile swath widths each release to complete one mile swath widths when two releases were made in a week. Initial release rates were planned to be at $250 \text{ flies/mi}^2/\text{wk.}$ ($655 \text{ flies/km}^2/\text{wk.}$). Depending upon the sterility rate observed at this level, release rates for later periods were to be maintained or adjusted upward.

Ancillary tests and methods, such as the release of marked flies were

planned if advisable (indeed, several such releases were made). Ecological data from weather stations in each zone were collected (by J. Mackley) throughout the test period and area. These will be analyzed in detail and reported upon later.

Results and Discussion

Egg mass collections:

The numbers of egg masses collected, reared, and sampled from sentinel sheep pens are shown in Table 1. The data are broken down by sheep pen, zone, and time period. The pre-release period lasted six weeks (April 25 - June 4), while the post-release period was divided into two phases, June 5 to July 4, and July 5 to August 10. During the pre-release period, several hundred egg masses were collected in each zone. Throughout the entire post-release period of about ten weeks (June 5 - August 10) about 500 egg masses were collected from each zone. For each of the nine collection totals (3 zones x 3 time periods each), between 62% and 82% of the egg masses collected were actually reared. The remainder could not be reared because either (1) the egg mass was sterile - ca. 2.5% of the total pre-release number and ca. 9% of the total post-release number; (2) the egg mass had hatched in the sheep wound prior to collection, thus precluding rearing a known pure culture; or (3) the egg mass succumbed to various handling procedures and died before reaching the rearing facility in Tuxtla-Gutierrez. It was assumed that the influences of (2) and (3) above were independent of the genetic characteristics of the native parents involved, or that at least their effects were unchanged in comparisons of pre - and post-release periods. This assumption seems reasonable, but, regardless, there was no way to know otherwise. Determining whether or not

factor (1) above is genotype independent is, of course, the crux of the whole field test. Natural sterility during the pre-release period might be expected to be genotype independent, but surplus, induced sterility caused by sterile released flies might not affect all genetic types equally.

As can be noted in Table 1, some sheep pens in each zone were 'hot' sites for collecting masses, while others were 'cold'. Some exceptional pens stayed 'hot' during all three time periods (eg. El Caribe in Zone A), others such as La Ilsa (Zone C) went from 'hot' to 'cold', and still others like La Reforma (Zone C) gradually became 'hotter'. For most pens, however, the numbers varied less dramatically. It is hoped that further understanding of the environmental peculiarities surrounding various pen and trap sites gained from recorded weather data will shed some light upon the reasons behind observed changes in egg mass collection number over time and space.

An indicator of relative population size is available from the index of average egg mass number per pen per day. Such data have been computed for each zone (Table 1) and time period and are graphed in Figure 2. Based upon this data, the screwworm population in Zone A would appear to have dropped steadily during the test period. The population in Zone B would appear to have peaked in the second time period (1st post-release) before dropping precipitously in the third time period (2nd post-release). The native population in Zone C started relatively high, then seemed to fall, before rising to its highest level at the end of the test. Indeed, the population in Zone C appeared to continue rising into the period of the V-81 strain evaluation which followed. The population changes in each zone far exceed the effects

caused by induced sterility (see Table 1 and discussion below). Though not included at this time, the data for collections of adults followed closely the pattern for egg masses, not surprisingly. Again, the adult data will be discussed in detail in a follow-up report. The apparent shifting of population peaks eastward over time, if real, may have been caused by either directed (eastward) migration of screwworm adults or delayed onset of optimal conditions for screwworm outbreaks in eastern parts of the test zone, or both. As we shall see from an examination of the sterility data below, native and/or released screwworm adults moved extensively in both directions into Zone B, making the second explanation above seem more likely.

Table 1 also includes the raw data for the numbers of larval samples collected by pen, zone, and time period. Approximately 80% of the egg masses reared were eventually sampled as third instar larvae, with some variation of course. Failure to adapt to the artificial meat media probably accounts for most of the lost egg mass groups - a feature commonly observed when some 'wild' strains are brought into the laboratory. The egg mass groups showed varying degrees of larval death, a fact which may be correlated with the karyotype (or chromosome constitution) of the larvae. Even if the latter were true, it was assumed that such effects were unchanged in comparing the different time periods of the test. After taking into account both the percentages of egg masses not reared and larval groups lost, roughly 60% of the egg masses collected could be analyzed genetically. Induced sterility comprised only a portion of the remaining ca. 40%; however it was a critical part since it was the only factor considered potentially genotype (karyotype) dependent.

Sterile fly releases and quality of strains:

Table 2 shows the data for the numbers of flies released, the release rates per week, and the emergence percentages (post-flight) for the two tested strains. Two release schedules were carried out during the nine week period (6/1 - 8/2). The first release of V-81 in Zone C came on June 5, and that of RGM-6 on June 6. The last releases for V-81 and RGM-6 came on July 30 and August 1, respectively. An initial release rate of 250 flies/mi.²/wk. for each strain was planned, but for several reasons this figure was never attained. First, instead of two releases per week only one was possible during each of the first two weeks for both strains. Second, it was discovered later, after an accurate topographic map became available to us, that the area of Zone C covered was actually over 800 sq. mi. (2,000 km²) rather than ca. 500 sq. mi. This factor, of course, diluted the density of V-81 flies released over Zone C. Third, even at the relatively low release rate desired, production problems of the RGM-6 strain quickly became evident. Lower pupal yield for RGM-6 resulted in a further decrease in its release rate. Fourth, the less than 100% adult emergence further reduced the numbers of both strains. The emergence numbers shown in Table 2 are averages of two boxes examined post-flight, except where rough figures are given the boxes were actually sampled pre-flight. Except for the final week of release, the emergence of RGM-6 was consistently lower than for V-81, thus reducing the release rate of the former even more severely. As can be seen the estimated number of released flies vacillated considerably for both strains, averaging between 100-125 flies/mi.²/wk. over the first four (for V-81) or five (for RGM-6) weeks. A gap of 12 days followed (6/29 - 7/10 for V-81,

6/30 - 7/11 for RGM-6) to allow a buildup of each strain and to make the changes in sheep pen locations mentioned earlier. A release rate of 500 flies/mi.²/wk. was planned but again the instances of only one release per wk. and less than desired production and emergence made achieving this goal impossible until the last one or two weeks of releases.

Information regarding the relative rearing capabilities of the V-81 and RGM-6 is largely qualitative at the time of this writing. Efforts will be made to secure quantitative data with respect to fecundity, fertility, egg hatch, larval size and mortality, and pupal yield for both tested strains and report on any such data in the follow-up report. However, the information available now on adult emergence and rearing qualities suggest that V-81 was adapting well to growth on artificial media while RGM-6 was adapting poorly. Adult emergence below 80% occurred often for RGM-6 and, when considered together with consistent problems in obtaining customary pupal yields for this strain, suggests comparatively poor viability of this strain. The effect, if any, upon the field performance of RGM-6 was not evident in lower sterility, as will be discussed below; however, perhaps RGM-6 would have realized even higher sterility had its viability been better. Comparatively poor rearing conditions vis-a-vis V-81, though a possibility, did not appear to be an important factor, if one at all. It is the opinion of the first author that the poor rearing quality of the RGM-6 strain was an inherent trait since production problems were evident from the time of its inception in the laboratory. Single egg mass lines, such as RGM-6, typically have

shown a wider range of adaptability to artificial media than have strains composed of multiple egg masses. This fact is not surprising considering the relative genetic potentials available. Some single egg mass lines may rear very well, others fairly well, and still others poorly. What is more important is that they appear to resist changes in their adaptability level much more so than do multiple egg mass lines, again probably because of a narrower genetic base to exploit. The RGM-6 line, selected in the Arriaga test because of its karyotype (by the Univ. of Texas researchers), just happened to be a relatively poor choice from a rearing standpoint. Reduction in viability due to inbreeding does not appear to be a satisfactory explanation because (1) as previously mentioned, some single egg mass lines have shown good rearing qualities, perhaps even excellent ones, in spite of any inbreeding and (2) multiple egg mass screwworm strains typically have had their components maintained as separate egg mass lines for the initial several generations - exactly the time when most of the amount of inbreeding was established. If adaptability to artificial rearing conditions were our only goal, then a multiple egg mass strain composed of even grossly genetically compatible lines would probably be the best. Of course, we are principally interested in the field, not factory, worthiness of released strains. Therefore, to the extent that a screwworm fly's genetic background can be implicated in its relative effectiveness in the field (esp. in mating), sterile release strains should be selected first on their genetic nature, and only second on their adaptability to artificial rearing conditions.

Egg mass sterility over time:

Table 3 shows the results for the numbers of egg masses collected and percent sterility during the entire Arriaga test. In the pre-

release period, between 1.7 and 3.1 percent sterility was observed for the three zones, though the majority of the sterile masses came in a two week period (5/11 - 5/24). The figures of 11.3% and 10% sterility obtained for Zones B and C during the week of 5/11 - 5/17 suggest some factor(s) besides just natural sterility was (were) operating. Handling and/or insecticide effects could have been involved but no concrete evidence was established. Sterility did drop back down to near zero, normal levels prior to the onset of releases. During the period of the lower release rate (6/6 to 6/29 in Zone A, 6/5 - 6/28 in Zone C), sterility in Zone A rose (to 13%) then fell to zero while in Zone C sterility rose (to 7.3%) after 1 week then dropped gradually. During the 12 da. gap period sterility was zero in Zone A, and near zero in Zone C. Interestingly, and disturbingly too, sterility in the middle 'control' zone was consistently higher than in either release zone. Migration of released sterile males of one or both strains and/or of native females mated to sterile males from the release zones into Zone B almost certainly took place (see Table 1 again). Sterility in the center of Zone B (around the pen, El Paraiso) began to increase after about $1\frac{1}{2}$ weeks and had 'caught up' with other pens by the time of the onset of the higher release schedule. The distinct pattern of decreasing sterility into Zone B from both release zones during the 1st post-release period strongly suggests migratory (or dispersal) influences. Though interesting in its own right, the evident considerable migratory powers of the screwworm fly in the wet, tropical Arriaga area - a matter of some doubt prior to the releases - caused a major problem in interpreting the genetic data. The control zone was set up to provide a base line

from which to measure changes in chromosome types. Only with such a control area can any such changes caused by the relative mating capabilities of the released sterile flies be clearly separated from other environmental effects causing similar changes. Several approaches discussed below were invoked to try to circumvent this problem and provide adequate, if less certain, genetic inferences. This problem of high dispersal rate surfaced again in the V-81 strain evaluation which will be discussed briefly further below and in more detail in the follow-up report when the field evaluation is concluded.

During the period of the second post-release (7/10-8/16) sterility rose, then fell, then rose again (to 30%) in Zone A. The latter sterility level was caused by a relatively sudden explosion of sterile egg masses between 8/12-8/14 almost two weeks after the last release. In Zone C, sterility jumped dramatically (to 22%) in the first week at the higher release rate, then dropped steadily throughout the remainder of the test. Sterility in Zone B patterned that of Zone A, i.e. it rose, then fell, then appeared to rise again at the end of the test. Over the last six weeks of the test sterility averaged 10% in Zone A, 5.8% in Zone B, and 9.9% in Zone C. It would appear that the efforts designed to reduce sterility in Zone B pens met with success, yet some influence of migration was still felt. For the whole test period, sterility in all three zones was about the same, i.e. 8-10%. With natural sterility approximately 2-3% based upon pre-release data, the sterile flies of either strain exerted an additional sterilizing influence of about 5-8%, on the whole. This figure could be misleading, however, since sterility reached higher levels at times and because the sterilizing influence exerted on some chromosome types could have been much higher than the effect upon other types.

Some of the data in Tables 1 and 3 were subjected to correlation analysis. First, release rate (Flies/mi.²/wk) was compared to observed sterility during the same week (no lag time), 1 week later, and 2 weeks later. In the instance

of no lag time for the 5 weeks of the lower release rate (6/1 - 7/5), no significant correlation was observed between weekly release rate and sterility for either strain. Similarly, no significant results were noted for no lag time during the 4 weeks of the higher release rate (7/6 - 8/2) or for the entire 9 weeks of releases (6/1 - 8/2). Correlations involving observed sterility one week later were all negative for the V-81 strain, and the value for lower rate releases was -0.88 ($p = 0.05$, $df = 3$). Comparable values for the RGM-6 strain were positive or negative, and non-significant. The negative correlations appear to have been caused by accidental coinciding of arbitrarily chosen weekly start and stop points with a regular pattern of release. A biological explanation cannot be ruled out, however. Correlations for sterility two weeks lagging were again all negative and non-significant for the V-81 strain; however correlations for RGM-6 releases yielded two notable positive values, 0.93 ($.05 < p < .10$, $df = 2$) for lower rate releases, and 0.88 ($p < .05$, $df = 7$) for the entire release period. These significant values may be accidental or artifactual, but they could also reflect the expected 1-2 week average lag time between mating and first oviposition in the field.

Another interesting correlation emerged from a comparison of total egg masses collected in a week's period and percent sterility observed. Considering the 9 week release period, (6/8 - 8/9), significant positive correlations were obtained for data from Zone A, $r = 0.88$ ($p < .01$, $df = 7$), from Zone B, $r = 0.59$ ($.05 < p < .1$, $df = 7$), and from Zone C, $r = 0.65$ ($.05 < p < .1$, $df = 7$). For all the data combined (27 values), a highly significant correlation of 0.63 ($p < .01$,

df = 25) was obtained (see Figure 3). This pattern appeared to continue into the period of the strain evaluation as well. At this time we can only speculate about the biological and/or physical factors contributing to this phenomenon. Could it be that if and when native females began to aggregate at oviposition sites the sterile released males succeed in sterilizing a larger proportion of them, on the average?

Chromosome Analysis

A total of 95 egg mass sibship groups have been analyzed involving 37 pre-release, 30 1st post-release, and 28 2nd post-release groups. A total of 31 groups were examined from Zone A (between 8 to 13 by period), 36 groups from Zone B (between 9 to 16 by period), and 28 groups from Zone C (between 8 to 11 by period). Of the 95 total groups, 16 yielded incomplete information for one or both native parents. The latter resulted from instances where complete genetic information could not be obtained from a minimum of five individuals, including two of each sex. However, since only cells yielding complete information were used, the genetic inferences made about the native parents, though incomplete, are still accurate. Roughly 5% of the groups examined for electrophoretic variation (over 300 in total so far) were each suspected as having been derived from a multiple egg mass - 2 or more egg mass groups mixed together. To avoid confusion in genetic interpretations, none of these groups were included in the chromosome analysis. Computer assisted analysis was performed on 23 pre-release groups plus the V-81 and RGM-6 release strains. Over 500 complete cells, each with 12 individual chromosomes (6 pairs), were measured using a highly accurate digital electronic planimeter.

The resulting numerical data was entered onto the computer according to egg mass group, sex, individual number, cell number, and slide coordinates. The numerical data itself consisted of short arm and long arm measurements for each of the 12 chromosomes per cell. The chromosomes were designated as X or Y sex chromosomes, or II, III, IV, V, and VI autosomes according to the convention established by Ellison (Univ. of Texas, personal communication) and applied by McInnis (1981, in press) and Richardson, et al (1981, in press). The screwworm, Cochliomyia hominivorax, in all samples examined to date, has had 6 pairs of chromosomes (Kaufman and Wasserman, 1957; Boyes, 1967; Boyes and Shewell, 1975). The X and Y sex chromosomes are the shortest pair, with males heterogametic (XY) and females homogametic (XX). Chromosome II is the longest chromosome with a centromere (primary constriction) located submetacentrically (somewhat off-center). Chromosome III is distinguished by a pair (except on one occasion) of brightly fluorescing spots located adjacent to the centromere. Chromosome IV is the most metacentric of the non-fluorescing autosomes. Chromosome V is submetacentric, yet shorter than chromosome II. Finally, chromosome VI is the most acrocentric of the autosomes. The ranges in length, arm ratio and fluorescent pattern for chromosomes found in the Arriaga test area are shown in Figures 4-7. More about them will be discussed later.

Visual and Computer assisted analysis:

Computer programs of the Statistical Analysis System (SAS) located at Texas A & M University analyzed the chromosome data. The objectives of the computer analysis were two-fold. First, it was hoped that subtle numerical analysis would confirm the visual analysis at the microscope level, and perhaps even detect variation

missed by visual methods alone. Second, accurate quantification of standard lengths and arm ratios, and especially the standard errors of these factors would allow one to assess the pattern of chromosome variation among native screwworms. Cells with complete chromosome information were scored visually for chromosome length, arm ratio, fluorescence, and banding patterns. Numerical analysis from the computer, in part, provided critical information on cell to cell variation of standard lengths and arm ratios within individuals. Since there should be no real genetic variation for these parameters within individuals, the observed variation is an error term attributable to human measurement error or apparent irregularities in chromosome condensation during the mitotic process. With practice and use of replicated measurements on each chromosome, variation attributable to human error was 5% or less. This included both the procedures of tracing over the chromosomes on prints and measuring them with the planimeter. The chromosomes were traced over while viewing the original glass slide chromosome preparation under the UV microscope. This procedure heightened the accuracy of the following chromosome measurements. The second factor in the error term, variations in long and/or short arm condensation during mitosis, was much larger than the first factor, as a rule. Arm ratios and standardized (or normalized) lengths for a particular chromosome could vary cell to cell by as much as 100% and 50%, respectively. This is why a minimum of five, and usually ten, cells were considered necessary to be able to type chromosomes accurately. With a sample size of five cells per individual, the coefficients of variation (standard deviation/mean) for standardized length and arm ratio were about 10% and 20%, respectively. Complete analyses of

variance tests comparing cell to cell (within individual), individual to individual (within a group), and group to group variations meant that chromosomal heterogeneity could be detected, including heterozygosity. Cluster and discriminant analyses procedures were also used to determine how closely related the egg mass groups were, and especially to note if distinct classes could be formed. Various 'tune-up' data from various non-Arriaga screwworm strains, were run through the system until it was operating smoothly.

Then a number of individuals from the RGM-6 and V-81 release strains were sampled and analyzed with the computer statistical package. For each strain, three pre-release and two post-release generations were examined (see Table 4). Some of the individuals in each strain did not yield complete chromosome information because unfortunately many of these larval samples were not collected properly (rapid freezing in liquid N₂ at the crawl-off stage). Cells with partial information were included only after visual and computer analysis (on complete cells only) together confirmed the chromosome types found in RGM-6 and V-81. As can be seen, RGM-6 was homozygous or homotypic for a single variant chromosome for each of the seven chromosome types (X, Y, II, III, IV, V, and VI). Since RGM-6 originated from a single egg mass presumably the native parents had been homozygous (or hemizygous in the case of the male's sex chromosomes) for the types listed in Table 4 and shown (along with others) in Figures 4-7. These chromosomes were the most common, or in some cases the only, types found in RGM-6's source population around Veracruz, Mex. based upon a limited number of samples analyzed in the summer of 1980. Those chromosome forms have been found in

other screwworm populations from the Mexican states of Colima, Sinaloa, and Tamaulipas. Of course, it is not possible to say that chromosomes of the same type are exactly the same genetically, indeed many differences in chromosome rearrangements (inversions, translocations), not to mention simple gene changes, could be present and would be undetectable with the available techniques. Therefore, each of the types listed in Tables 4-7 really represent a variant class having at least one member. It is clear from the analysis of RGM-6 samples over time that reasonably high, if not absolute, purity was maintained both before and during the test. The V-81 strain provided chromosome heterogeneity, including heterozygous individuals, for three of the seven kinds of chromosomes. Three each of X, Y, and V chromosomes were detected out of 34 individuals sampled. The remaining four chromosomes, II, III, IV, and VI, were monomorphic (i.e. were of a single type). For the X and V chromosomes the most frequent variants (80% or more) were also the ones found in RGM-6. Of the variant Y chromosomes in V-81, two were roughly equally frequent (based upon a relatively ^{SMALL} number of males) and one of these two was the type found in RGM-6. Therefore, with the exception of one Y chromosome, RGM-6 and V-81 looked very much alike karyotypically. Of course there could have been substantial genetic differences between the two strains that were undetected with the available techniques. Based upon the limited number of individuals examined, no significant deviations from random mating expectations were noted in the V-81 line. That is, heterozygotes of various kinds (X and V chromosomes) were observed in roughly the expected frequencies. Since V-81 was created through reciprocal forced crosses between

pairs of separate egg mass lines it was not surprising to observe chromosomal heterozygotes. However, such heterozygotes in nature may be rare (McInnis, in press) or absent (Richardson, et al, in press). The presence of heterozygotes at, or near, random mating levels long after the final forced crosses were carried out means that a significant proportion of heterotypic matings (crosses between flies carrying unlike types) continued to occur, albeit under artificial conditions.

The combination of visual and computer assisted analysis of the 23 pre-release egg mass groups (divided among all 3 zones) having measured chromosome data revealed the following:

First, the types of chromosomes resolvable by computer analysis matched those discernable from visual analysis alone with the following exceptions. Where discrete variations were detected for bright (X and III chromosomes) or faint (II chromosome) fluorescence, computer analysis would fail to detect such types unless standard length or arm ratio statistics were also diagnostic (eg. the cases of X-3 and X-7). Again, where a secondary constriction (band) variation was detected visually on an X chromosome, the computer could not detect it (note type X-6, Figure 4). Computer analysis of variance revealed statistically significant (F-test, $p < .05$) variation between individuals within a group and between groups for II chromosome standard lengths and arm ratios. No such variation was scored visually. To date the computer's 'find' has not been retraced to locate the group or groups involved; however this will be done through careful examination of the data or by sequential addition of groups to the computer's data base until a significant F-statistic is obtained.

Second, a significant level of within-group chromosome heterogeneity (including individual heterozygosity) was obtained. Table 5 includes the data from all 95 groups examined but the pattern was similar for the 23 pre-release groups analyzed by computer. A discussion about the inferred genotypes of the native parents will be made further below.

Third, based upon the Arriaga samples analyzed thus far, no recognizable

pattern emerged to suggest the existence of two or more discrete (or diagnostic) karyotype sets representing distinct, yet coexisting (sympatric) screwworm populations (gamodemes). The genotypes of the inferred native parents, various X chromosomes were associated with a number of different Y chromosomes and these with many of the different autosomal types. Where sample size was sufficiently large, no statistically significant deviation from expected values was obtained for the associations of two or more of the seven chromosome types (X, Y, II, III, IV, V, VI). It is not necessary to have heterozygotes to rule out the existence of distinct gamodemes, but having heterozygotes means that the uniqueness of a particular set of non-homologous chromosomes is violated in a single individual. A consequence of the multiple gamodeme hypothesis is that the genetic (karyotypic) differences between groups would be expected to be relatively large or small depending upon whether a comparison between gamodeme clusters or within a gamodeme cluster was made, respectively. Measures of squared genetic distance based upon the criteria of arm ratios (12/cell) and standard lengths (12/cell) were available after discriminant analysis of most of the pre-release groups. Between any two groups A and B, a squared distance, D^2 , was computed equal to the matrix product of the differences in the vectors of arm ratios and standard lengths for groups A & B with the inverse of the covariance matrix:

$$D^2 = (\bar{X}_I - \bar{X}_J)' \cdot \text{COV.}^{-1} \cdot (\bar{X}_I - \bar{X}_J) \quad .$$

Because of the heterogeneity involving differences between X and Y chromosomes, only females (21 egg mass groups) or only males (15 egg mass groups) were compared. The results are shown in Figures 8 and 9 for females and males, respectively. The genetic distance values are only relative, of course, since they depend entirely upon the size of the numerical figures entered as arm ratios or standard lengths. The ordinates of each graph represent the numbers of different pair combinations falling within corresponding genetic intervals. It is clear from both graphs that

proportionally few combinations yielded very small or very large genetic distance values - just the opposite of what is predicted by a multiple gamodeme model involving quantitative determiners. The latter would predict a rather concave or bowl-shaped frequency distribution. The skewing to the right is caused largely by the fact that the genetic distances are squared. The distribution of the square roots of those values would be much more symmetrical about the apex. The observed and the latter distributions are highly consistent with what would be predicted under the alternative to a multiple gamodeme model, i.e. the single gamodeme or polymorphic variation model. Genetic distances (even squared distances) should be largely moderate in size with few extremely large or small values, as observed in Figures 8 and 9. This is because differences between groups are for one, or at most a few chromosomes rather than for all seven chromosome forms when changes occur under the multiple gamodeme model. Though the final data are not yet complete, there would appear to be an expected strong positive correlation between the number (and magnitude) of chromosomal changes between groups and the calculated genetic distance. However, it is also clear that spuriously large genetic distances result when sample size per group is small, especially when only one individual or fewer than five cells are involved. All five cases of squared genetic distance above a value of 32 (Figure 9) and many of those distances above a value of 20 (both figures) came from comparisons with groups of very small sample size. This effect reduces much of the difference in distributions for males and females, though the male Y chromosome could still have a significant impact. Of no small value are the comparative genetic distances between either of the release strains, RGM-6 or V-81, and the remaining F1 groups. For female comparisons (Figure 8), the average squared genetic distances for V-81 and RGM-6 were 7.53 (± 0.70) and 5.99 (± 0.88), respectively. As we shall

see below, these moderate values are not surprising, considering the chromosome compositions of pre-release groups in Zones A and C. For Male comparisons (Figure 9), the corresponding distances for V-81 and RGM-6 were $7.76 (\pm 1.37)$ and $12.54 (\pm 1.25)$, respectively. These values are significantly different ($p < .01$) and may relate to the fact that V-81 had three Y chromosomes (instead of only one) with which to increase overall compatibility with native males. Obviously, the genetic distance model is only sketchy at this time and more refinements are necessary, especially to make it more comprehensive by including qualitative factors such as fluorescence patterns.

Fourth, computer assisted analysis provided mean and standard error data for arm ratios and standard lengths to compare with purported gamodeme data claimed by the University of Texas collaborators. The summary data made available by those researchers appeared in their annual cooperative agreement report of 1980. Identical laboratory techniques were used to obtain their data and ours, including the method in which summary data were prepared. Therefore, the results are entirely comparable. For each of the 25 computer analyzed troupes (including RGM-6 and V-81), at best only a poor fit could be made to any of the nine (Type G has been deleted) gamodeme types currently recognized by the University of Texas (UT) group. Indeed, what is critical is that data from several Arriaga test groups could each be fitted (albeit poorly, and for different reasons) to several UT designated gamodemes. Arriaga test groups containing heterozygotes could not be made to fit at all, since each gamodeme is considered to be homozygous for a distinct variant of each chromosome pair. Under such a multiple gamodeme model, heterozygotes or hybrids between gamodemes would produce recognizable differences for each chromosome pair in all F_1 offspring. Heterozygotes found in the Arriaga test samples were considered to be heterozygous for at most a few chromosomes (including the celebrated triploid group thought by UT to contain some intergamodemic hybrids). What causes the striking differences in

interpretation between the UT and USDA researchers on this subject? It is the opinion of this first author that most of the reason stems from differences in the sizes of within group variation (#cells/individual and # individuals/sibship) measured by each research group. It is very clear from our own analysis that when within group sample size was restricted, (some of the pre-release groups were this way, partly intentionally) the between group genetic distance is spuriously large. Increasing the number of complete cells examined per individual reduces the standard errors for quantitative factors such as arm ratios and standard lengths and heightens the provability of detecting differences between the members of a chromosome pair, i.e. heterozygosity. Examining more individuals per sibship also means increasing the probability of detecting heterozygosity, especially if the baseline is small to begin with. Increasing the within group sample allows visual analysis to provide a greater conservative check upon computer quantitative analysis, and vice-versa. Significant genetic differences for heterozygous individuals should be consistent from cell to cell and chromosome heterogeneity within a group should be consistent with Mendelian inheritance (unless a multiple egg mass origin can be invoked).

The remaining 72 Arriaga test F_1 groups were only analyzed visually, in part because of the very good correspondence between visual and computer analysis on the 23 pre-release groups, and in part due to the limited time available to increase sample size without compromising within group standards. Although it is possible that multiple gamodemes existed in the Arriaga test area and were missed, at least one of these - the only one recognized by this group's work - would have been polymorphic in nature. The single gamodeme model is not inconsistent with assortative mating, however, as will be noted below.

Polymorphic chromosomes:

The recognized single gamodeme chromosome variants are shown in Figures 4 - 7. The changes over time and area (zone) for these variants, and the meaning

of such changes will be discussed further, below. Seven distinct X, eight Y, two II, three III, two IV, three V, and two VI chromosomes were observed. Of the 27 total variants over half (15) were X or Y types. This is noteworthy, especially as the sex chromosomes are the smallest ones. Though it is probable that additional variants would be found after examining more samples, these would be few in number judging from the rate of adding new variants experienced at the end of the sampling period. As can be seen in Figure 4, the various X chromosome types differ from each other in standard length (expressed as a proportion of the whole genome) arm ratio (short arm/long arm), and fluorescence and banding pattern. Approximate standard deviations are also included. The variations were probably produced following a series of chromosomal duplications, deletions, and possibly translocations or inversions in a couple of original types. Five of the X types (X-1, X-2, X-4, X-5, X-6) were observed previously in 27 egg mass groups collected around Aldama (ca. 1500 km²), Tamaulipas, Mexico during 1979 (McInnis, in press). Several of those same five types have been found in the Mexican states of Sinaloa, Colima, and Veracruz within the last two years. The surprisingly large number of Y chromosome variants (Figure 5) again differ from each other in length and in arm ratio, though all were non-fluorescent and lacked secondary banding. Four of these, Y-2, Y-3, Y-4, and Y-6, were found in Aldama and elsewhere as well. The extremely small, dot-like Y-1, and the very long Y-8 (longer than most X's) were exciting finds. The two II chromosomes in Figure 6 were similar in length and in arm ratio, but differed in that one type has reliable, if only faint, fluorescence around the centromere. The rare fluorescent form had been observed earlier in Sinaloa. Two of the three III chromosomes (Figure 6) have two brightly fluorescent spots at the centromere (III-1 and III-3) but differ in length. The third type, III-2, deserves some mention for a couple of reasons. First, it was observed only once (collected at La Ilsa on 4/27), in heterozygous form, and is the first III chromosome observed by the first author to possess only one bright spot at the centromere. Some fluorescence may be present on the short arm,

but if so, it is only a small amount and perhaps even variable. Second, after bringing this unusual group to the attention of the UT researchers, they found that their duplicate sample contained two bonafide triploid individuals. None of the latter had been detected in the USDA sample out of six larvae yielding good preparations. The UT researchers have postulated that the triploid individuals arose through fertilization of parthenogenetically produced eggs, others of which went unfertilized to produce diploid females via parthenogenesis. However, after careful examination of the group, including one of the triploid individuals, it would appear to the first author that a freak developmental mutation producing diploid eggs is much more likely to be the reason for triploidy than parthenogenesis. The latter cannot be ruled out, though, and if true it would pose an obvious problem to the Commission's eradication program. The remaining autosomal variants are shown in Figure 7. Two IV variants were observed, though the longer form (IV-1) was found in only two larvae from separate groups and, unfortunately, only incomplete information was available on the genotypes of the native parents of these larvae. This rare long form had been observed previously in Sinaloa, though it was absent from Aldama collections. Of the V chromosomes, V-1 and V-3 were noted earlier in Aldama and Sinaloa, and all three were found in Veracruz. Both VI types had been observed earlier in Aldama and Sinaloa, and the long form was much more frequent on these occasions. The variants of the autosomal pairs IV through VI are perhaps interrelated through various structural mutations.

Genotypic Frequencies:

Table 5 shows the observed and random mating expected genotype frequencies for the inferred native parents in each zone over the entire test period. For Zone A over the entire test period a significant excess of homozygotes was noted for chromosomes II and V only. (chi. sq. test p values $< .01$.) When the pre-release and two post-release periods are examined separately, however, several striking

trends are noted. Non-significant homozygote excess for the X chromosome shifts toward near-significant heterozygote excess during the 2nd post-release period. This is caused by an increase in heterozygotes of the X-1:X-3 sort to a level of 67% (6/9) of native female parents, and a corresponding decrease in the frequency of X-1 homozygotes. We shall see below how this trend relates to an apparent positive assortative mating effect exerted by the X-1 chromosome type found in RGM-6. Changes in chromosome V in Zone A over time was equally striking. Highly significant homozygote excess (19 out of 20 genotypes observed, $p < .01$) shifted to a random mating expected number of heterozygotes during the 2nd post-release period. Of these seven heterozygotes, five were of the V-1:V-2 homozygotes. Since RGM-6 carried only the V-1 type (barring contamination), the changes in chromosome V could have been caused by negative assortative mating. The changes occurring in Zone B need to be accounted for first however, as will be done below. During the pre-release period in Zone A, 4 (out of 20) heterozygous parents were inferred, (3 of these X-1:X-3 females) whereas over 14(14.35) individuals heterozygous for at least one chromosome were expected. This difference is highly significant statistically. In the 1st post-release period a similarly significant though less marked deficiency of heterozygous individuals was noted. In the 2nd post-release period a dramatic change occurred. Due mostly to the X-1:X-3, and V-1:V-2 heterozygotes, along with five II, III, and VI chromosome heterozygotes, close to the expected number of heterozygous individuals (for at least one chromosome) was obtained. Finally, the numbers of homotypic matings (between like types) were not significantly different from random mating expectations for the X chromosome. Yet an excess of such matings appeared to shift to a slight deficiency by the time of the 2nd post-release period. A statistically significant ($p < .01$) excess of homotypic matings was noted for chromosomes II and V, especially for the latter in the pre-release period. At that time, nine out of ten homotypic matings were observed, including several of the V-2:V-2 x V-2:V-2 sort.

Based upon the data in Table 5, the genotypic frequencies in Zones B and C showed significant excesses of homozygotes for chromosomes II and V, and chromosome V, respectively. Unlike for Zone A, no significant changes in heterozygosity were noted over time. In both zones, there was a highly significant deficiency of inferred parents heterozygous for at least one chromosome. Also, the pattern of a higher than expected frequency of homotypic matings, especially for chromosome V, was again evident.

The consistently significant patterns of homozygote and homotypic mating excesses meant that random mating among the native screwworms of Arriaga had not occurred, at least until sterile flies were dropped. As noted above, RGM-6 may have caused the shift toward heterozygosity and heterotypic matings observed in Zone A during the latter part of the test. What may have caused the deviations from random mating expectations? Heterozygote deficiency could be caused by a combination of several factors including (1) positive assortative mating, (2) inbreeding, (3) heterozygote inviability, and (4) sampling errors. The evident great dispersal powers of the native screwworm fly in Arriaga, at least in the presence of sterile flies, would tend to rule out (2) above as being a major factor. However, to the extent that some segment of the screwworm population shows little mobility and tends to mate with near relatives, inbreeding could be a partial cause of heterozygote deficiency. Various forms of selection could be operating against heterozygotes in the wild, however, if this factor (3) were a major cause it would have to be occurring primarily before the third instar larval stage because a strong deficiency of heterozygotes was detected then. Indeed, it will be recalled that, consistently, some 40% of the egg masses collected did not yield any samples. It is possible that offspring of heterotypic matings comprised a large proportion of this unsampled group. Also, quite often heterozygous larvae appear at less than expected frequencies within

an egg mass sample (McInnis, unpublished data from several studies), suggesting a selective effect operating against them (though perhaps only on artificial media). Thus, strong selection against heterozygotes in nature may be a major factor. However, it alone cannot explain the genotypic and mating pattern changes noted above in Zone A or the dramatic chromosome frequency changes to be discussed below. Sampling errors of various kinds, though undefined, also are very likely to have been consistent throughout the time period of the test. Therefore, though sample size was by no means large, it would appear to have been quite adequate to rule out a significant effect of sampling error upon the observed genotypic patterns. The remaining potential factor, positive assortative mating will now be considered.

Evidence for Assortative Mating:

The frequencies of the various chromosomal variants are listed in Table 6 by chromosomal type, Zone, and time period. Observed numbers and the associated proportions are indicated along with expected values computed according to the method (for X-1) discussed in the Appendix. The latter are based upon the degree of chromosome frequency changes observed in a given time period in the 'control' zone, Zone B. Because a significant amount of sterility was recorded in Zone B, a genetic interpretation is confounded. Since the sterility in Zone B was almost surely caused by both V-81 and RGM-6 release strains, their effects in Zone B could either complement or neutralize each other. Where sterile strain effects might be expected to compound one another, i. e. for identical chromosomes II, III, IV, and VI, the changes in Zone B attributable to sterile flies will be similar to those in Zones A and C. Resulting comparative differences and tests of significance will be conservative. On the other hand, for chromosomes X, Y, and V, some neutralizing effect in Zone B could result if some of the polymorphic variants found in V-81 exerted opposing influences. This potential neutralizing effect could counter some environmentally caused changes and enhance the statistical differences between

Zone B and primarily Zone A. The effect upon Zones B and C comparisons would be expected to be a conservative one, again since the postulated neutralizing forces would be occurring in both zones.

In order to analyze the effect, if any, of each chromosome separately, the frequencies of the various chromosomal types had to be given absolute, rather than relative, values. If only relative frequencies were considered, a real effect upon even a single type would cause bogus changes in all of the remaining types of that particular chromosome. In order to estimate absolute frequencies, the egg mass collection data from Table 1 was used. Then, by computing the proportional change in absolute level realized over a given time interval in Zone B, one could compute the assumed identical changes expected for Zones A and C. The method of calculating the expected values for the X-1 type in the Appendix was applied for all such values listed in Table 6. After the expected values were known, chi-square tests of significance were performed. Values significant at the 5% and 1% levels are indicated by one and two asterisks, respectively.

For the X chromosomes, statistically significant deviations from expected values were obtained for types X-1 and X-4 in the first and/or second post-release periods in Zone C. A similar, though not quite statistically significant change was observed in Zone A for type X-1. The changes in relative frequencies in each zone for type X-1 can be seen in Figure 10. The relative changes in the two release zones do appear to be greater than the change in Zone B. The chi-square tests in RGM-6 and by far the most frequent one in V-81 (ca. 90%). Another notable change in X chromosomes was that type X-3 increased in Zones A and C, while only fluctuating in Zone B. In relating the observed changes to the chromosome types found in the release strains, one notices a striking pattern. The frequencies of the three X chromosomes found in V-81 all declined in Zone C relative to expected values, while other types registered absolute gains. A similar effect was noted in Zone A. It is unfortunate that RGM-6 and V-81 were so alike karyotypically so that the

possible confounding effects in Zone B weren't greater. Quantitative measures of relative mating capabilities for chromosome types found in the two release strains are shown in Table 7. These are computed as equal to the proportional change from the expected number calculated earlier and listed in Table 6. Positive values indicate that fewer than the expected number were observed, suggesting some sterilizing effect of released flies. A maximum value of 1 is possible, i.e. when the number observed is zero. If the numbers of observed and expected are equal, the calculated effect of the sterile fly is appropriately zero. Negative relative mating abilities (up to $1-\infty$) occur when observed values are greater than expected. The size of the negative number may be inversely related to the abilities of types less affected by sterile flies to expand their numbers, perhaps into temporarily less competitive niche space. Undefined values (listed as undef.) result from division by expected values of zero. Many of these values would probably have been negative if sample size had been larger. This would result from non-zero observed numbers for those particular types in the associated pre-release period. Of course, the calculated values in Table 7 cannot be taken at face value since experimental errors probably exist and only some of the positive or negative values are statistically significant. The chromosome types found in the two release strains are indicated in the left margin alongside the particular variant name.

The Y chromosome also showed some interesting trends. In Zone A, the type found in RGM-6, Y-4, decreased significantly in accordance with an 'indirect' positive assortative mating model (See Figure 11). That is native females would deprive native males carrying the Y-4 type by mating predominantly with RGM-6 males. The significant decrease in the Y-5 type in Zone A, if not an accident caused by small sample size in Zone B, represented another indirect female effect, though negative in this instance. Type Y-6 vacillated sharply in frequency, while types Y-2 and Y-7 increased. The Chi-Square test statistics for these last types were

undefined, however, and suggest again the need to sample the pre-release period more until some low level of these can be detected. In Zone C, two of the Y chromosomes found in V-81, Y-3 and Y-4, decreased over time though not significantly. The effect of the remaining V-81 Y type, Y-6, was neutral based upon the data.

For chromosome II no significant effects were noted in either zone, though a trend toward decreasing the RGM-6 type II-1 was noted in Zone A. Again, no significant trends were observed for chromosomes III, IV, or VI in either Zone A or Zone C.

Chromosome V provided some significant effects, and was the only autosome to do so. Not surprisingly, no significant changes were detected in Zone C since the V-81 strain released there contained all three variants detected in the field and in roughly the same proportions. In Zone A an interesting and highly statistically significant effect was observed. The only variant found in RGM-6 (unless it was contaminated and eluded detection) was type V-1. This form increased dramatically, while type V-II decreased sharply, all by the time of the 1st post-release. These effects suggest a strong negative assortative mating effect was operating for type V-1 in Zone A. As discussed earlier, the statistical difference for chromosome V in Zone A could have been enhanced by the neutralizing effect of V-81 flies in Zone B. However, the magnitude of the changes in Zone A suggest that this is probably not the only factor. The relative mating capacity model described is one of two undergoing further refinement in order to better quantify screwworm mating abilities.

As we have seen, three of the seven chromosome types, X, Y, and V, registered some significant effects upon the native fly population in Arriaga. Environmental effects, though confounded by sterility in Zone B, were at least reasonably controlled against. The residuals were, for the most part, conservative estimates of the effects of the sterile strains. It is noteworthy that positive, negative, and

neutral effects for different variants of these three chromosomes were all observed, based upon the compositions of the release strains. The data suggest that a wide genotypic array of mating abilities exists in the screwworm. It is clear from the genotypic distribution that no evidence for complete mating barriers existed in Arriaga. This fact is corroborated by earlier studies such as (1) laboratory mating (including multiple choice) experiments indicating no significant mating barrier among screwworms, albeit in the laboratory (McInnis and Whitten, unpubl. data), (2) the two successfully completed field evaluations of the earlier DE-9 and the current Sinaloa strain - both forced-cross derived strains that were considerably different chromosomally from their target populations (McInnis, unpubl. data), and supported by (3) the remarkable success of the eradication program when sterile fly releases were properly executed in all respects and the sterile strain was not highly factory adapted. However, the strong pattern of homotypic matings coupled with the positive correlations noted in chromosome frequency changes above do indicate that mating preferences do exist for some chromosome types. Looked at in another way, various mating thresholds exist which are overcome with varying degrees of facility. For example, based upon the genotypic data and the comparative ease with which the common X chromosome type, X-1, was reduced by both V-81 and RGM-6, it would appear that flies of the X-1 type may be 'favorite' mating partners, having a low mating threshold. It is perhaps of no small coincidence that this X-1 type has been found in every screwworm population studied thus far by these authors and has always been at, or near to, the top in relative frequency. Such a generalist role, to a lesser degree could also be ascribed to males carrying the Y-4 type - also highly commonplace. Therefore, though all native screwworms have, so far, possessed surmountable mating barriers at some density of viable sterile flies, the efficiency of a release program might increase materially if released strains were more genetically compatible with target popu-

lations. The morphological, ethological, or chemical reasons contributing to relative mating ability (or susceptibility) have, of course, not been elaborated upon as yet. Also, from a genetic standpoint we have only dealt with gross chromosomal or karyotypic variation. Variation at the gene level in the form of several major genes or possible coadapted gene complexes may play crucial roles in affecting fly viability (including mating ability). Such a screwworm program consideration as where or how to develop new factory strains should recognize our great ignorance about locally adapted screwworm populations and attempt to let the fly itself guide us whenever possible. Perhaps, in time, we can learn enough to reverse roles and guide the fly -- to extinction.

Conclusions

Concerning the primary objectives of the test:

1. Native screwworm flies in the Arriaga area were found to demonstrate non-random, assortative mating patterns in varying degrees (weak or strong) and kinds (positive or negative).
2. Both the narrow (single egg mass) strain and the broader (multiple egg mass) strain demonstrated some assortative mating tendencies depending upon the chromosome type considered.

Other information gained:

3. Native screwworm flies in the Arriaga area demonstrated polymorphic chromosome variation characteristic of a single gamodeme.
4. The single egg mass strain demonstrated relatively poor viability under mass rearing conditions. This fact does not indict all single egg mass lines, it only means that relative adaptability to artificial rearing conditions must be considered in selecting potential factory strains.
5. The native population in Arriaga during April to August was relatively

large based upon egg masses collected and capable of rapid expansion.

6. The native and/or sterile flies were capable of dispersing at rates comparable to those experienced in drier, temperate regions. The native adults, however, could barely be trapped using the customary lures.
7. Release rates of 100 - 500 flies per square mile per week induced about 10% sterility in the native population. This was sufficient to cause significant genetic changes over time.

Recommendations for the future of the Eradication Program

1. Due to the demonstrable existence of assortative mating patterns among screwworms, it has become more critical to investigate the genetics of target native populations and sterile, release strains. For the reason of at least greater efficiency, if not for achieving population control at all, released strains should be selected to provide the greatest genetic compatibility with target native populations. The latter should be sampled and studied as much ahead of encountering sterile flies as possible to allow for necessary or advisable changes to be made.
2. Future strains developed for factory production should be derived as much as possible from a local area (ca. 500 - 1500 sq. miles) to help ensure (though not guarantee) a genetically compatible set of component egg mass lines. Also, future strains should be sampled as much as possible from areas likely to have sterile releases made of those strains.
3. To allow for the possibility of genetic incompatibility among sympatrically derived lines selected for new strain development, forced crosses among flies should be avoided in favor of allowing the flies themselves to choose mates.
4. To the extent possible, several factory strains should be maintained and released separately or in combination depending upon the target population. Each strain should be chosen in order to supplement the other(s) in terms of geographical origin and perhaps genetic composition. This will provide for greater program flexibility and reduce the chances of encountering incompatible, resistant native populations.

Status report on the V-81 strain evaluation

The last fly release during the earlier Arriaga test was made on August 1. By August 24th the production of V-81 had been increased sufficiently in the Methods and Development section of the screwworm facility in Tuxtla-Gutierrez to begin aerial releases. Approximately 1200 sq. miles of the earlier Zones B and C in Arriaga were chosen to receive a dose of 1500 flies per sq. mile per week. After three weeks of releases, sterility had reached 25% (avg. for the third week) but did not go higher thereafter. Because of the demonstrably large dispersal powers of the screwworm fly in Arriaga coupled with a probable lack of adequate insulation for sentinel sheep pens, the shape of the tested area has been changed to minimize the potential effect of migrating fertile females entering the test area. Only after the effect of this change is known will the possibility of genetic incompatibility between V-81 and the current native population in Arriaga be clear. A report discussing the final outcome of the V-81 strain evaluation will be made soon after its completion in October or early November.

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Appendix

Calculation of expected chromosome frequencies

Example :

Objective: To determine the expected proportion and number of med., fl., acrocentric (1 band) X chromosomes for the 1st Post-release period in Zone A.

1. Calculate the population levels in terms of egg masses collected per pen day for the relevant zones and time periods (in this case Zones A and B for the Pre-release and 1st Post-release periods). These values are available from Table 1.

	<u>Zone A</u>	<u>Zone B</u>
Pre-release	1.99	1.25
1st Post-release	1.67	2.10

2. Assuming each egg mass collected arose from distinct or separate male and female parents (i.e. no multiple ovipositions) or that exceptions to this rule were equally frequent in all three zones, then each egg mass contributed 3 X chromosomes, 1 Y chromosome, and 4 autosome sets to the sample genetic pool. In this case, the number of X chromosomes per pen day may be calculated by multiplying 3 times each of the numbers in (1) above, yielding the following:

	<u>Zone A</u>	<u>Zone B</u>
Pre-release	5.97	3.75
1st Post-release	5.01	6.03

Appendix cont'd.

3. For the particular chromosome desired (in this case, type X-1), calculate the number of such chromosomes on a per pen day basis. This required use of the observed proportions listed in Table as follows:

	<u>Zone A</u>	<u>Zone B</u>
Pre-release	$(5.97)(.80) = 4.78$	$(3.75)(.5) = 1.88$
1st Post-release	$(5.01)(.71) = 3.56$	$(6.03)(.54) = 3.26$

4. Compute the proportional change in the absolute 'population' level of X-1 in Zone B from the Pre-release to the 1st Post-release periods, i.e. $3.26/1.88 = 1.73$. (In other instances if the divisor is zero, the quotient becomes undefined. Then the Pre-release period in the relevant zone (Zone A here) may function as an uncorrected control. This is equivalent to saying there was no change in Zone B, i.e. the quotient = 1).
5. Multiply the proportional change obtained in (4) above by the relevant Pre-release 'population' level of the X-1 type, i.e. $(4.78)(1.73) = 8.29$. This value is the expected number of X-1 chromosomes for Zone A during the 1st Post-release period, whereas 3.56 were actually observed.
6. After completing the above steps for the remaining six X chromosomes and summing all seven values obtained at step (5) above, a figure of 10.29 X chromosomes per pen day results. Therefore, the figure of 8.29 X -1 chromosomes per pen day represents an expected proportion of 0.81 of the total. Since there were a total of 24 X chromosomes sampled in Zone A during the 1st Post-release period, 19.4 X -1 chromosomes (24×0.81) were expected. The observed proportion and number were 0.71 and 17, respectively.

Table 1. Numbers of egg masses collected, reared, and sampled from sentinel sheep pens.

ARRIAGA TEST - Pre-release Period (4/25 to 6/4)

<u>Zone</u>	<u>Sheep Pen</u>	<u># Egg masses collected</u>	<u># and (%) reared</u>	<u># sampled</u>	<u>% sampled of # reared</u>	<u>% sampled of # collected</u>
A	Las Pilas (LPA)	18	13 (72)	9	(69)	(50)
	Los Horcones (LHA)	92	60 (65)	48	(80)	(52)
	El Encanto (EEA)	89	61 (69)	51	(84)	(57)
	El Caribe (ECA)	97	90 (93)	70	(78)	(72)
	San Francisco (SFA)	<u>111</u>	<u>88 (79)</u>	<u>73</u>	<u>(83)</u>	<u>(66)</u>
		407	312 (77)	251	(80)	(62)

407 masses per (41 days X 5 pens) = 1.99 egg masses/pen day.

B	Santa Cruz (SCB)	61	58 (95)	47	(81)	(77)
	La Sonia (LSB)	48	35 (73)	29	(83)	(60)
	El Paraiso (EPB)	80	65 (81)	58	(89)	(73)
	El Brazil (EBB)	44	34 (78)	26	(76)	(59)
	San Pedro (SPB)	<u>23</u>	<u>18 (78)</u>	<u>15</u>	<u>(83)</u>	<u>(65)</u>
		256	210 (82)	175	(83)	(68)

256 masses per (41 days X 5 pens) = 1.25 egg masses/pen day.

C	La Ilsa (LIC)	112	72 (64)	54	(76)	(48)
	Vado Ancho (VAC)	40	28 (70)	23	(82)	(58)
	Los Carlos (LCC)	93	51 (55)	45	(88)	(48)
	La Reforma (LRC)	28	24 (86)	18	(75)	(64)
	El Vergel (EVC)	<u>80</u>	<u>45 (56)</u>	<u>34</u>	<u>(76)</u>	<u>(43)</u>
		353	220 (62)	174	(79)	(49)

353 masses per (41 days X 5 pens) = 1.72 egg masses/pen day.

Table 1 cont'd.

ARRIAGA TEST - 1st Post Release Period (6/5 to 7/4)

Zone	Sheep Pen	# Egg masses collected	# and % Sterility	# and (%) Reared	# Sampled	% Sampled of # reared	% Sampled of # collected
A	Las Pilas ((LPA)	13	1 (7.7)	6 (46)	5	83	39
	El Caribe (ECA)	121	8 (6.6)	81 (67)	73	90	60
	Los Horcones (LHA)	61	9 (14.8)	47 (77)	42	89	69
	El Encanto (EEA)	28	2 (7.1)	23 (82)	19	83	68
	San Francisco (SFA)	<u>28</u>	<u>3 (10.7)</u>	<u>18 (64)</u>	<u>16</u>	<u>89</u>	<u>57</u>
		251	23 (9.2)	185 (74)	155	84	62
251 egg masses per (30 days X 5 pens) = 1.67 egg masses/pen day.							
B	Santa Cruz (SCB)	30	3 (10.0)	20 (67)	16	80	53
	La Sonia (LSB)	39	3 (7.6)	21 (54)	20	95	51
	El Paraiso (EPB)	66	4 (6.1)	52 (79)	46	88	70
	El Brazil (EBB)	99	13 (13.1)	79 (80)	60	76	61
	San Pedro (SPB)	<u>81</u>	<u>12 (14.8)</u>	<u>55 (68)</u>	<u>44</u>	<u>80</u>	<u>54</u>
		315	35 (11.1)	227 (72)	186	82	59
315 egg masses per (30 days X 5 pens) = 2.10 egg masses/pen day.							
C	La Ilsa (LIC)	9	0 (0.0)	9 (100)	7	78	78
	Vado Ancho (VAC)	6	1 (16.7)	5 (83)	5	100	83
	Los Carlos (LCC)	69	4 (5.8)	51 (74)	41	80	59
	La Reforma (LRC)	52	2 (3.8)	39 (75)	36	92	69
	El Vergel (EVC)	<u>7</u>	<u>0 (0.0)</u>	<u>5 (71)</u>	<u>5</u>	<u>100</u>	<u>71</u>
		143	7 (4.9)	109 (76)	94	86	66

143 egg masses per (30 days X 5 pens) = 0.95 egg masses/pen day.

Table 1 cont'd.

ARRIAGA TEST - 2nd Post-release Period (7/5 to 8/10)

Zone	Sheep Pen	# Egg masses collected	# and % Sterility	# and (%) Reared	# Sampled	% Sampled of # reared	% Sampled of # collected
A	Las Pilas (LPA)	37	4 (10.8)	21 (57)	14	67	38
	El Caribe (ECA)	70	1 (1.4)	56 (80)	48	86	69
	Los Horcones (LHA)	97	9 (9.3)	54 (56)	39	72	40
	El Ocuilapa (EOA)	9	0 (0.0)	5 (56)	5	100	56
	San Martin (SMA)	<u>4</u>	<u>1 (25.0)</u>	<u>3 (75)</u>	<u>3</u>	<u>100</u>	<u>75</u>
		217	15 (6.9)	139 (64)	109	78	51
217 egg masses per (37 days X 5 pens) = 1.17 egg masses/pen day.							
B	Santa Cruz (SCB)	29	2 (6.9)	18 (62)	15	83	52
	La Sonia (LSB)	42	3 (7.1)	25 (60)	16	64	38
	El Paraiso (EPB)	47	4 (8.5)	37 (79)	35	95	74
	El Brazil (EBB)	24	1 (4.2)	15 (63)	12	80	50
	San Pedro (SPB)	<u>38</u>	<u>0 (0.0)</u>	<u>35 (92)</u>	<u>17</u>	<u>49</u>	<u>45</u>
		180	10 (5.6)	130 (72)	95	73	53
180 egg masses per (37 days X 5 pens) = 0.97 egg masses/pen day.							
C	Los Mendez (LMC)	11	3 (27.2)	7 (64)	6	86	55
	Vado Ancho (VAC)	68	9 (13.2)	40 (59)	34	85	50
	Los Carlos (LCC)	75	12 (18.1)	48 (64)	45	94	60
	La Reforma (LRC)	138	11 (8.0)	88 (64)	81	92	59
	El Vergel (EVC)	<u>29</u>	<u>1 (3.4)</u>	<u>19 (66)</u>	<u>17</u>	<u>89</u>	<u>59</u>
		321	36 (11.2)	202 (63)	183	91	57

321 egg masses per (37 days X 5 pens) = 1.74 egg masses/pen day.

Table 2. Numbers of flies released, release rate per week, and percent emergence (post-flight) for the V-81 and RGM-6 release strains.

V-81							RGM-6					
Week	# Releases	# Boxes	# Pupae	% Emergence	# Flies per mi ²	(km ²)	# Releases	# Boxes	# Pupae	% Emergence	# Flies per mi ²	(km ²)
6/1-6/7	1	1120	78,400	94	88	(226)	1	1108	77,560	ca. 85	ca. 94	(241)
6/8-6/14	1	936	65,520	93	74	(188)	1	847	59,290	89	95	(242)
6/15-6/21	2	2099	146,930	95	167	(428)	2	1636	114,520	79	162	(415)
6/22-6/28	2	1524	106,680	ca.90	115	(294)	1	1164	81,480	ca. 85	ca.124	(317)
6/29-7/5	-	-	-	-	-	-	1	492	34,420	ca. 90	ca.55	(142)
7/6-7/12	1	913	127,820	93	254	(649)	1	592	82,880	ca. 60	ca.128	(329)
7/13-7/19	1	724	101,360	ca.75	162	(415)	1	687	96,180	ca. 70	ca.174	(445)
7/20-7/26	2	1804	252,560	96	517	(1323)	1	238	33,320	82	71	(181)
7/27-8/2	2	1420	198,800	84	356	(911)	2	1425	199,500	ca. 93	479	(1227)
Avg. (7/6-8/2):						322 (824)	Avg. (7/6-8/2): 213 (545)					

Table 3. Egg masses collected and percent sterility during the Arriaga test.

Week	Zone A				Zone B				Zone C			
	Tot. #	# Fer- tile	# Ster- ile	% Ster- ility	Tot. #	# Fer- tile	# Ster- ile	% Ster- ility	Tot. #	# Fer- tile	# Ster- ile	% Ster- ility
1. 4/25,4/26	19	(19)	-	-	13	(13)	-	-	3	(3)	-	-
2. 4/27-5/3	110	(110)	-	-	69	(69)	-	-	53	(53)	-	-
3. 5/4-5/10	76	(75)	(1)	1.3	63	(63)	-	-	79	(79)	-	-
4. 5/11-5/17	81	(78)	(3)	3.8	53	(47)	(6)	11.3	40	(36)	(4)	10.0
5. 5/18-5/24	84	(81)	(3)	3.6	45	(43)	(2)	4.4	119	(112)	(7)	5.9
6. 5/25-5/31	33	(33)	-	-	17	(17)	-	-	48	(48)	-	-
7. 6/1-6/7	31	(29)	(2)	6.5	12	(12)	-	-	19	(19)	-	-
8. 6/8-6/14	92	(80)	(12)	13.0	88	(75)	(13)	14.8	41	(38)	(3)	7.3
9. 6/15-6/21	84	(75)	(9)	10.7	127	(109)	(18)	14.2	34	(32)	(2)	5.9
10. 6/22-6/28	33	(33)	-	-	54	(52)	(2)	3.7	29	(28)	(1)	3.4
11. 6/29-7/5	26	(26)	-	-	35	(33)	(2)	5.7	46	(45)	(1)	2.1
12. 7/6-7/12	40	(37)	(3)	7.5	16	(14)	(2)	12.5	72	(56)	(16)	22.2
13. 7/13-7/19	62	(55)	(7)	11.3	17	(17)	-	-	66	(58)	(8)	12.1
14. 7/20-7/26	27	(26)	(1)	3.7	62	(59)	(3)	4.8	60	(54)	(6)	10.0
15. 7/27-8/2	39	(38)	(1)	2.6	50	(48)	(2)	4.0	77	(72)	(5)	6.5
16. 8/3-8/9	43	(40)	(3)	7.0	32	(29)	(3)	9.4	25	(24)	(1)	4.0
17. 8/10-8/16	30	(21)	(9)	30.0	10	(9)	(1)	10.0	63	(63)	-	-
Pre- Release totals: 410 (4/25-6/4)	410	(403)	(7)	1.7	264	(256)	(8)	3.0	352	(341)	(11)	3.1
Totals (all zones):	1026	(1000)	(26)	2.5								
Post- Release totals: 500 (6/5-8/16)	500	(453)	(47)	9.4	499	(453)	(46)	9.2	522	(479)	(43)	8.2
	910				763				874			
Totals (all zones):	1521	(1385)	(136)	8.9								

Table 4 Chromosome frequencies for the release strains, RGM-6 and V-81.

		<u>RGM-6</u>							
<u>Generations Sampled</u>	<u># Inds. Sampled</u>	<u>CHROMOSOME</u>							
		X	Y	II	III	IV	V	VI	
4,5,9,11,13	34	1. Med., fl. acroc. (1 band) X-1	1. Med., subme- tac. Y-4	1. Subme- tac., non-fl. II-1	1. Med. Sub- metac. (2 spots) III-1	1. Short, metac. IV-1	1. Short, subme- tac. V-1	1. Short acroc. VI-1	
		57(100%)	10(100%)	46(100%)	47(100%)	45(100%)	45(100%)	45(100%)	

		<u>V-81</u>							
		<u>CHROMOSOME</u>							
		X	Y	II	III	IV	V	VI	
9,10,11,14,16	34	1. Med., fl. acroc. (1 band) X-1	1. Med., subme- tac. Y-4	1. Subme- tac., non-fl. II-1	1. Med., Sub- metac. (2 spots) III-1	1. Short, metac. IV-1	1. Short, subme- tac. V-1	1. Short acroc. VI-1	
		42(89.4%)	7(41.2%)	58(100%)	56(100%)	56(100%)	48(80%)	54(100%)	
		2. Med., non- fl. acroc. (1 band) X-2 2(4.3%)	2. Long subme- tac. Y-6 9(52.9%)				2. Med., subme- tac. V-2 4(6.7%)		
		3. Long, fl. acroc. (1 band) X-4 3(6.3%)	3. Short, subte- loc. Y-3 1(5.9%)				3. Long, subme- tac. V-3 8(13.3%)		

Table 5

VARIANT #	DATE	DESCRIPTION	AMOUNT	BALANCE
1	1/1/19	Initial deposit	100.00	100.00
2	1/15/19	Withdrawal	25.00	75.00
3	2/1/19	Deposit	50.00	125.00
4	2/15/19	Withdrawal	10.00	115.00
5	3/1/19	Deposit	75.00	190.00
6	3/15/19	Withdrawal	30.00	160.00
7	4/1/19	Deposit	40.00	200.00
8	4/15/19	Withdrawal	15.00	185.00
9	5/1/19	Deposit	60.00	245.00
10	5/15/19	Withdrawal	20.00	225.00
11	6/1/19	Deposit	80.00	305.00
12	6/15/19	Withdrawal	25.00	280.00
13	7/1/19	Deposit	90.00	370.00
14	7/15/19	Withdrawal	35.00	335.00
15	8/1/19	Deposit	100.00	435.00
16	8/15/19	Withdrawal	40.00	395.00
17	9/1/19	Deposit	110.00	505.00
18	9/15/19	Withdrawal	45.00	460.00
19	10/1/19	Deposit	120.00	580.00
20	10/15/19	Withdrawal	50.00	530.00
21	11/1/19	Deposit	130.00	660.00
22	11/15/19	Withdrawal	55.00	605.00
23	12/1/19	Deposit	140.00	745.00
24	12/15/19	Withdrawal	60.00	685.00
25	1/1/20	Deposit	150.00	835.00
26	1/15/20	Withdrawal	65.00	770.00
27	2/1/20	Deposit	160.00	930.00
28	2/15/20	Withdrawal	70.00	860.00
29	3/1/20	Deposit	170.00	1030.00
30	3/15/20	Withdrawal	75.00	955.00
31	4/1/20	Deposit	180.00	1135.00
32	4/15/20	Withdrawal	80.00	1055.00
33	5/1/20	Deposit	190.00	1245.00
34	5/15/20	Withdrawal	85.00	1160.00
35	6/1/20	Deposit	200.00	1360.00
36	6/15/20	Withdrawal	90.00	1270.00
37	7/1/20	Deposit	210.00	1480.00
38	7/15/20	Withdrawal	95.00	1385.00
39	8/1/20	Deposit	220.00	1605.00
40	8/15/20	Withdrawal	100.00	1505.00
41	9/1/20	Deposit	230.00	1735.00
42	9/15/20	Withdrawal	105.00	1630.00
43	10/1/20	Deposit	240.00	1870.00
44	10/15/20	Withdrawal	110.00	1760.00
45	11/1/20	Deposit	250.00	2010.00
46	11/15/20	Withdrawal	115.00	1895.00
47	12/1/20	Deposit	260.00	2155.00
48	12/15/20	Withdrawal	120.00	2035.00
49	1/1/21	Deposit	270.00	2305.00
50	1/15/21	Withdrawal	125.00	2180.00
51	2/1/21	Deposit	280.00	2460.00
52	2/15/21	Withdrawal	130.00	2330.00
53	3/1/21	Deposit	290.00	2620.00
54	3/15/21	Withdrawal	135.00	2485.00
55	4/1/21	Deposit	300.00	2785.00
56	4/15/21	Withdrawal	140.00	2645.00
57	5/1/21	Deposit	310.00	2955.00
58	5/15/21	Withdrawal	145.00	2810.00
59	6/1/21	Deposit	320.00	3130.00
60	6/15/21	Withdrawal	150.00	2980.00
61	7/1/21	Deposit	330.00	3310.00
62	7/15/21	Withdrawal	155.00	3155.00
63	8/1/21	Deposit	340.00	3505.00
64	8/15/21	Withdrawal	160.00	3345.00
65	9/1/21	Deposit	350.00	3695.00
66	9/15/21	Withdrawal	165.00	3530.00
67	10/1/21	Deposit	360.00	3890.00
68	10/15/21	Withdrawal	170.00	3720.00
69	11/1/21	Deposit	370.00	4090.00
70	11/15/21	Withdrawal	175.00	3915.00
71	12/1/21	Deposit	380.00	4295.00
72	12/15/21	Withdrawal	180.00	4115.00
73	1/1/22	Deposit	390.00	4505.00
74	1/15/22	Withdrawal	185.00	4320.00
75	2/1/22	Deposit	400.00	4720.00
76	2/15/22	Withdrawal	190.00	4530.00
77	3/1/22	Deposit	410.00	4940.00
78	3/15/22	Withdrawal	195.00	4745.00
79	4/1/22	Deposit	420.00	5165.00
80	4/15/22	Withdrawal	200.00	4965.00
81	5/1/22	Deposit	430.00	5395.00
82	5/15/22	Withdrawal	205.00	5190.00
83	6/1/22	Deposit	440.00	5630.00
84	6/15/22	Withdrawal	210.00	5420.00
85	7/1/22	Deposit	450.00	5870.00
86	7/15/22	Withdrawal	215.00	5655.00
87	8/1/22	Deposit	460.00	6115.00
88	8/15/22	Withdrawal	220.00	5895.00
89	9/1/22	Deposit	470.00	6365.00
90	9/15/22	Withdrawal	225.00	6140.00
91	10/1/22	Deposit	480.00	6620.00
92	10/15/22	Withdrawal	230.00	6390.00
93	11/1/22	Deposit	490.00	6880.00
94	11/15/22	Withdrawal	235.00	6645.00
95	12/1/22	Deposit	500.00	7145.00
96	12/15/22	Withdrawal	240.00	6905.00
97	1/1/23	Deposit	510.00	7415.00
98	1/15/23	Withdrawal	245.00	7170.00
99	2/1/23	Deposit	520.00	7690.00
100	2/15/23	Withdrawal	250.00	7440.00

Table 5 (cont'd)

Chromosome

VARIANT

II

1	2
OBS.(EXP.)	OBS.(EXP.)

ZONE

VARIANT #	1	A	44 (40.7)	4 (10.6)
		B	54 (50.8)	2 (8.8)
		C	48 (47.1)	3 (4.8)
	2	A		4 (0.7)
		B		4 (0.4)
		C		1 (0.1)

VARIANT

III

1	2	3
OBS.(EXP.)	OBS.(EXP.)	OBS.(EXP.)

ZONE

VARIANT #	1	A	50 (50.0)	0 (-)	2 (2.0)
		B	60 (60.0)	0 (-)	0 (-)
		C	45 (43.4)	1 (0.9)	4 (7.6)
	2	A		0 (-)	0 (-)
		B		0 (-)	0 (-)
		C		0 (0)	0 (0)
	3	A			0 (0)
		B			0 (-)
		C			2 (0.3)

Table 5 (cont'd)

Chromosome

IV

		VARIANT #	
VARIANT #	ZONE	1	2
		OBS.(EXP.)	OBS.(EXP.)
1	A	52 (52)	0 (-)
	B	60 (60)	0 (-)
	C	52 (52)	0 (-)
2	A		0 (-)
	B		0 (-)
	C		0 (-)

V

		VARIANT #		
VARIANT #	ZONE	1	2	3
		OBS.(EXP.)	OBS.(EXP.)	OBS.(EXP.)
1	A	34 (28.5)	6 (14.6)	3 (5.4)
	B	41 (31.9)	4 (21.5)	0 (-)
	C	33 (29.8)	4 (11.0)	4 (3.7)
2	A		7 (1.9)	0 (1.4)
	B		13 (3.9)	0 (-)
	C		5 (1.1)	0 (0.7)
3	A			2 (0.2)
	B			0 (-)
	C			0 (0.1)

VI

		VARIANT #	
VARIANT #	ZONE	1	2
		OBS.(EXP.)	OBS.(EXP.)
1	A	51 (51.0)	1 (1.0)
	B	59 (59.0)	1 (1.0)
	C	49 (49.0)	3 (3.0)
2	A		0 (0)
	B		0 (0)
	C		0 (0)

Table 6 . Chromosome frequencies in the separate zones during pre- and post-release periods,

Chromosome type	Zone A			Zone B			Zone C		
	Pre-release (4/25-6/4) Observed # (prop)	Post-release		Pre-release (4/25-6/4) Observed # (prop)	Post-release		Pre-release (4/25-6/4) Observed # (prop)	Post-release	
		I	II		I	II		I	II
		(6/5-7/4)	(7/5-8/10)		(6/5-7/4)	(7/5-8/10)		(6/5-7/4)	(7/5-8/10)
		Observed # (prop)	(Expected #, prop)		Observed # (prop)	(Expected #, prop)		Observed # (prop)	(Expected #, prop)
A. X Variants									
1) Med., fl. acroc. (1 band) X-1	28 (.80)	17 (.71)	9 (.33)	22 (.50)	15 (.54)	6 (.22)	18 (.75)	19 (.61)**	8 (.31)*
		19.4 (.81)	14 (.52)					24.8 (.80)	13.9 (.58)
2) Med., non-fl. acroc. (1 band) X-2	0 (-)	0 (-)	0 (-)	1 (.02)	1 (.04)	1 (.04)	1 (.04)	1 (.03)	1 (.04)
		0 (-)	0 (-)					2.3 (.07)	3.4 (.14)
3) Med. -L, acroc. (1 band) X-3	7 (.20)	4 (.17)	16 (.60)	15 (.34)	7 (.25)	15 (.56)	0 (-)	10 (.32) ¹	14 (.54) ¹
		3.3 (.14)	13 (.48)					0 (-)	0 (-)
4) Long, fl., acroc. (1 band) X-4	0 (-)	3 (.12)	0 (-)	4 (.09)	0 (-)	2 (.07)	5 (.21)	1 (.03)	1 (.04)**
		1.4 (.05)	0 (-)					4 (.13)	6.7 (.28)
5) Long, fl., acroc. (2 bands) X-5	0 (-)	0 (-)	2 (.07) ¹	0 (-)	4 (.14)	3 (.11)	0 (-)	0 (-)	2 (.08) ¹
		0 (-)	0 (-)					0 (-)	0 (-)
6) Long, non-fl., acroc. (1 band) X-6	0 (-)	0 (-)	0 (-)	1 (.02)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
		0 (-)	0 (-)					0 (-)	0 (-)
7) V. long, fl., acroc. (1 band) X-7	0 (-)	0 (-)	0 (-)	1 (.02)	1 (.04)	0 (-)	0 (-)	0 (-)	0 (-)
		0 (-)	0 (-)					0 (-)	0 (-)

Note: * indicates statistical significance at the 5% (p=.05) level.

** indicates statistical significance at the 1% (p=.01) level.

1 indicates that Chi-Square test yielded an undefined value.

Table 6 cont'd.

Chromosome type	Zone A			Zone B			Zone C		
	Pre-release	Post-release		Pre-release	Post-release		Pre-release	Post-release	
	(4/25-6/4)	I	II	(4/25-6/4)	I	II	(4/25-6/4)	(6/5-7/4)	(7/5-8/10)
	Observed # (prop)	Observed # (prop)	(Expected #, prop)	Observed # (prop)	Observed # (prop)	(Expected #, prop)	Observed # (prop)	Observed # (prop)	(Expected #, prop)
B. Y variants									
1) V. short, submetac. Y-1	0 (-)	1 (.13) ¹	1 (.10)	0 (-)	2 (.20)	0 (-)	0 (-)	0 (-)	0 (-)
		0 (-)	0 (-)					0 (-)	0 (-)
2) Short, metac. Y-2	0 (-)	0 (-)	3 (.30) ¹	0 (-)	2 (.20)	0 (-)	0 (-)	3 (.27) ¹	2 (.25) ¹
		0 (-)	0 (-)					0 (-)	0 (-)
3) Short, subteloc. Y-3	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	1 (.14)	0 (-)	0 (-)
		0 (-)	0 (-)					0.7 (.08)	(.18)
4) Med., submetac Y-4	5 (.50)	5 (.62)	0 (-)**	8 (.57)	4 (.40)	6 (.67)	5 (.71)	3 (.27)	2 (.25)**
		3.7(.46)	4.3(.43)					4.2 (.47)	6.6(.82)
5) Med., acroc. Y-5	2 (.20)	0 (-)	0 (-)**	1 (.07)	0 (-)	2 (.22)	0 (-)	0 (-)	0 (-)
		0 (-)	4.4(.44)					0 (-)	0 (-)
6) Long, submetac Y-6	1 (.10)	0 (-)**	3 (.30) ¹	2 (.14)	2 (.20)	0 (-)	1 (.14)	3 (.27)	1 (.13) ¹
		3.7(.46)	0 (-)					4 (.45)	0 (-)
7) Long, acroc. Y-7	1 (.10)	2 (.25) ¹	3 (.30)	3 (.21)	0 (-)	1 (.11)	0 (-)	2 (.18) ¹	2 (.25) ¹
		0 (-)	0.4(.04)					0 (-)	0 (-)
8) V. long, submetac. Y-8	1 (.10)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	1 (.13) ¹
		0.6(.08)	0.9(.09)					0 (-)	0 (-)
C. #2 variants									
1) Submetac., non-fl. II-1	46 (1.00)	28 (.88)	28 (.78)	54 (.93)	36 (.86)	32 (.89)	31 (.97)	39 (.93)	33 (.97)
		32 (1.0)	36 (1.0)					39.5(.94)	32.3(.95)
2) Submetac., lgt. fl. (2 spots) II-2	0 (-)	4 (.12) ¹	8 (.22) ¹	4 (.07)	6 (.14)	4 (.11)	1 (.03)	3 (.07)	1 (.03)
		0 (-)	0 (-)					2.5(.06)	1.7(.05)

Table 6 cont'd.

Chromosome type	Zone A			Zone B			Zone C		
	Pre-release	Post-release		Pre-release	Post-release		Pre-release	Post-release	
	(4/25-6/4)	I	II	(4/25-6/4)	I	II	(4/25-6/4)	I	II
	Observed #(prop)	(6/5-7/4)	(7/5-8/10)	Observed #(prop)	(6/5-7/4)	(7/5-8/10)	Observed #(prop)	(6/5-7/4)	(7/5-8/10)
		Observed	Observed	Observed	Observed	Observed	Observed	Observed	Observed
		(Expected	(Expected	(Expected	(Expected	(Expected	(Expected	(Expected	(Expected
		#,prop)	#,prop)	#,prop)	#,prop)	#,prop)	#,prop)	#,prop)	#,prop)
D. #3 variants									
1) Med., submetac. (2 fl. spots) III-1	46 (1.00)	32 (1.00)	34 (.94)	58 (1.00)	38 (1.00)	36 (1.00)	31 (.97)	36 (.86)	32 (.94)
		32 (1.00)	36 (1.0)					41.2(.98)	32.6(.96)
2) Med., submetac. (1 fl. spot) III-2	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	1 (.03)	0 (-)	0 (-)
		0 (-)	0 (-)					0.8(.02)	1.4 (.04)
3) Short, submetac. (2 fl. spots) III-3	0 (-)	0 (-)	2 (.06) ¹	0 (-)	0 (-)	0 (-)	0 (-)	6 (.14) ¹	2 (.06) ¹
		0 (-)	0 (-)					0 (-)	0 (-)
E. #4 variants									
1) Short, metac. IV-1	44 (.96)	32 (1.00)	36 (1.00)	57 (.98)	38 (1.00)	36 (1.00)	32 (1.00)	42 (1.00)	34 (1.00)
		32 (1.00)	36 (1.00)					42 (1.00)	34 (1.00)
2) Long, metac. IV-2	2 (.04)	0 (-)	0 (-)	1 (.02)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
		0 (-)	0 (-)					0 (-)	0 (-)
F. #5 variants									
1) Short, submetac. V-1	27 (.59)	31 (.97)**	25 (.69)**	49 (.84)	26 (.68)	20 (.59)	26 (.81)	30 (.71)	23 (.68)
		21 (.40)	11.1 (.31)					28.2(.67)	19.7(.58)
2) Med., submetac. V-2	14 (.30)	1 (.03)**	7 (.19)**	9 (.16)	12 (.32)	14 (.41)	4 (.13)	12 (.29)	9 (.26)
		17.3(.54)	20.9(.58)					12 (.29)	11.6(.34)
3) Long, submetac V-3	5 (.11)	0 (-)	4 (.12)	0 (-)	0 (-)	0 (-)	2 (.06)	0 (-)	2 (.06)
		1.9 (.06)	4.0 (.11)					1.8 (.04)	2.7 (.08)

Table 6 cont'd.

Chromosome type	Zone A			Zone B			Zone C		
	Pre-release (4/25-6/4) Observed # (prop)	Post-release		Pre-release (4/25-6/4) Observed # (prop)	Post-release		Pre-release (4/25-6/4) Observed # (prop)	Post-release	
		I	II		I	II		I	II
		(6/5-7/4)	(7/5-8/10)		(6/5-7/4)	(7/5-8/10)		(6/5-7/4)	(7/5-8/10)
		Observed # (prop)	Observed # (prop)	Observed # (prop)	Observed # (prop)	Observed # (prop)	Observed # (prop)	Observed # (prop)	Observed # (prop)
			(Expected #, prop)						(Expected #, prop)
G. #6 variants									
1) Short, acroc. VI-1	46 (1.00)	32 (1.00)	35 (.97)	57 (.98)	37 (.97)	36 (1.00)	31 (.97)	42 (1.00)	32 (1.00)
		32 (1.00)	36 (1.00)					39.9 (0.95)	33.3 (.98)
2) Long, acroc. VI-2	0 (-)	0 (-)	1 (.03) ¹	1 (.02)	1 (.03)	0 (-)	1 (.03)	0 (-)	2 (.06)
		0 (-)	0 (-)					2.1 (.05)	0.7 (.02)

Table 7 Estimated relative mating capacities of the RGM-6 and V-81 release strains with native chromosome types.

Chromosome type	Estimated change (Relative mating ability = (Expected # - Observed #)/Expected, Table 6)			
	RGM-6 (Zone A releases)		V-81 (Zone releases)	
	<u>Post-Release I</u>	<u>Post-Release II</u>	<u>Post-Release I</u>	<u>Post-Release II</u>
A. X				
1. X-1: (RGM-6, V-81)	0.13	0.36	0.23**	0.41*
2. X-2: (V-81)	-	-	0.56	0.71
3. X-3:	-0.21	-0.23	(undef.)	(undef.)
4. X-4: (V-81)	-1.14	-	0.75	0.85**
5. X-5	-	(undef.)	-	(undef.)
6. X-6	-	-	-	-
7. X-7	-	-	-	-
B. Y				
1. Y-1:	(undef.)	(undef.)	-	-
2. Y-2:	-	(undef.)	(undef.)	(undef.)
3. Y-3: (V-81)	-	-	1.00	1.00
4. Y-4: (RGM-6, V-81)	-0.35	1.00**	0.29	0.70
5. Y-5:	-	1.00**	-	-
6. Y-6: (V-81)	1.00**	(undef.)	0.25	(undef.)
7. Y-7:	(undef.)	-6.5	(undef.)	(undef.)
8. Y-8:	1.00	1.00	-	(undef.)

Note: * or ** indicates value associated with a $p < .05$ or $p < .01$ chi. sq. test (Table 6).

Table 7 (cont'd.)

Chromosome type	<u>RGM-6 (Zone A releases)</u>		<u>V-81 (Zone C releases)</u>	
	<u>Post-Release I</u>	<u>Post-Release II</u>	<u>Post-Release I</u>	<u>Post-Release II</u>
C. II				
1. II-1: (RGM-6,V-81)	0.13	0.22	0.01	-0.02
2. II-2:	(undef.)	(undef.)	-0.20	0.41
D. III				
1. III-1: (RGM-6,V-81)	0	0.06	0.13	0.02
2. III-2:	-	-	1.00	1.00
3. III-3:	-	(undef.)	(undef.)	(undef.)
E. IV				
1. IV-1: (RGM-6,V-81)	0	0	0	0
2. IV-2:	-	-	-	-
F. V				
1. V-1: (RGM-6,V-81)	-1.42 ^{**}	-1.25 ^{**}	-0.06	0.26
2. V-2: (V-81)	0.91 ^{**}	0.67 ^{**}	0	0
3. V-3: (V-81)	1.00	0	1.00	-
G. VI				
1. VI-1: (RGM-6,V-81)	0	0.03	-0.05	-
2. VI-2:	-	-	1.00	-

Figure 1

ARRIAGA FIELD TEST AREA

Sheep Pens

Zone A

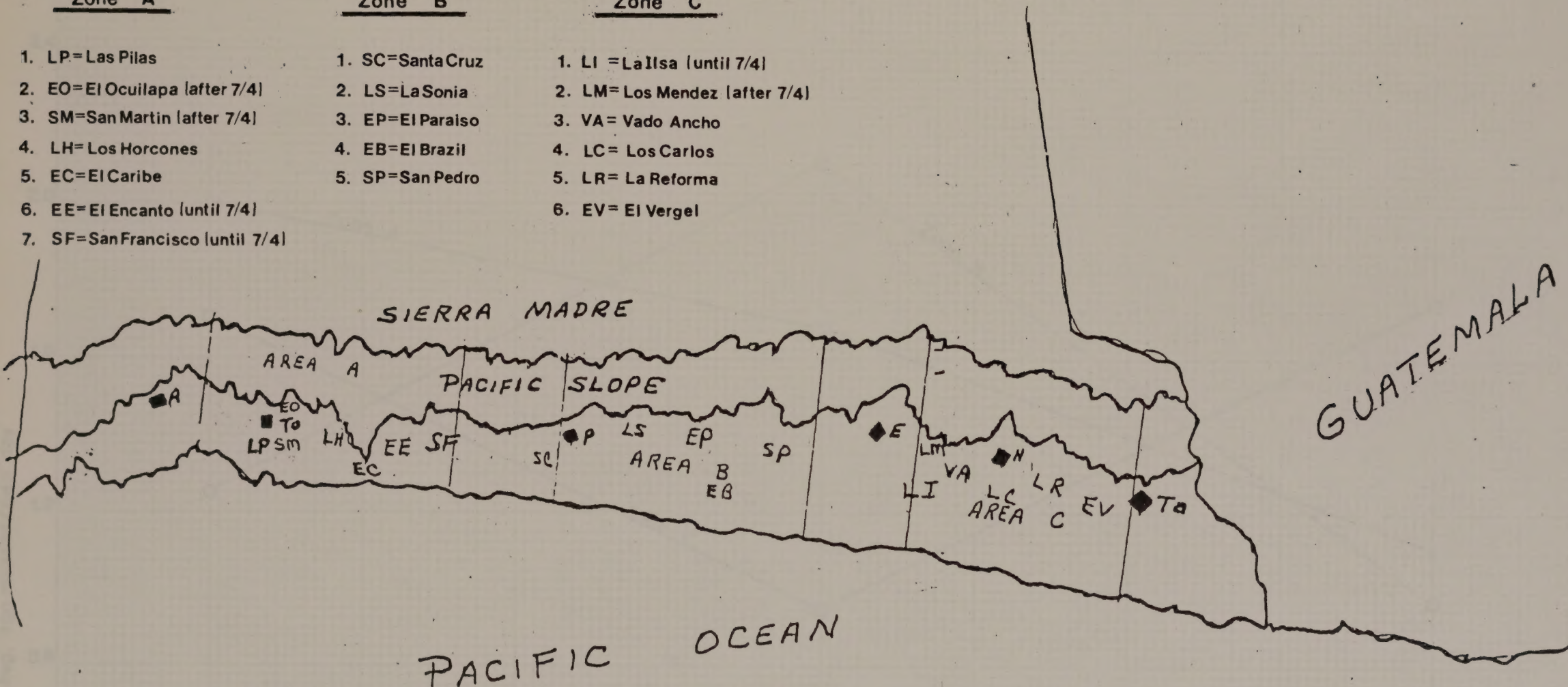
Zone B

Zone C

1. LP= Las Pilas
2. EO=El Ocuilapa (after 7/4)
3. SM=San Martin (after 7/4)
4. LH= Los Horcones
5. EC=El Caribe
6. EE=El Encanto (until 7/4)
7. SF=San Francisco (until 7/4)

1. SC=Santa Cruz
2. LS=La Sonia
3. EP=El Paraiso
4. EB=El Brazil
5. SP=San Pedro

1. LI = La Ilsa (until 7/4)
2. LM= Los Mendez (after 7/4)
3. VA= Vado Ancho
4. LC= Los Carlos
5. LR= La Reforma
6. EV= El Vergel



A = ARRIAGA,
 To = TONALA
 P = PIJINIAPAN
 E = ESQUINTLA
 H = HUIXTLA
 T = TAPACHULA

0 50
 1 INCH = 16 MILES

14°
 92°

Figure 2

ARRIAGA FIELD TEST

Population sizes based upon egg masses collected

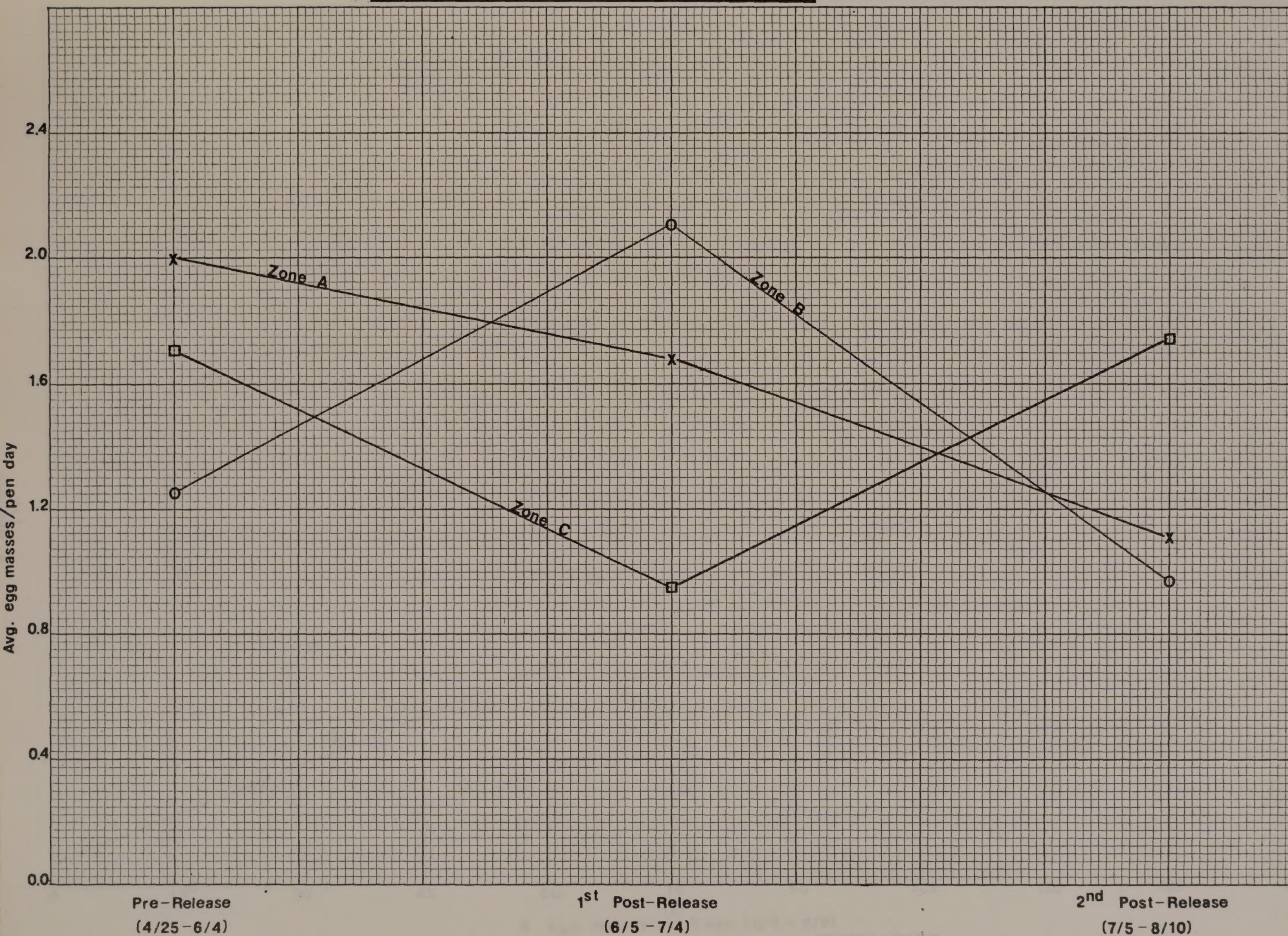


Figure 3

ARRIAGA FIELD TEST

Egg masses collected vs. percent sterility during the Arriaga test.

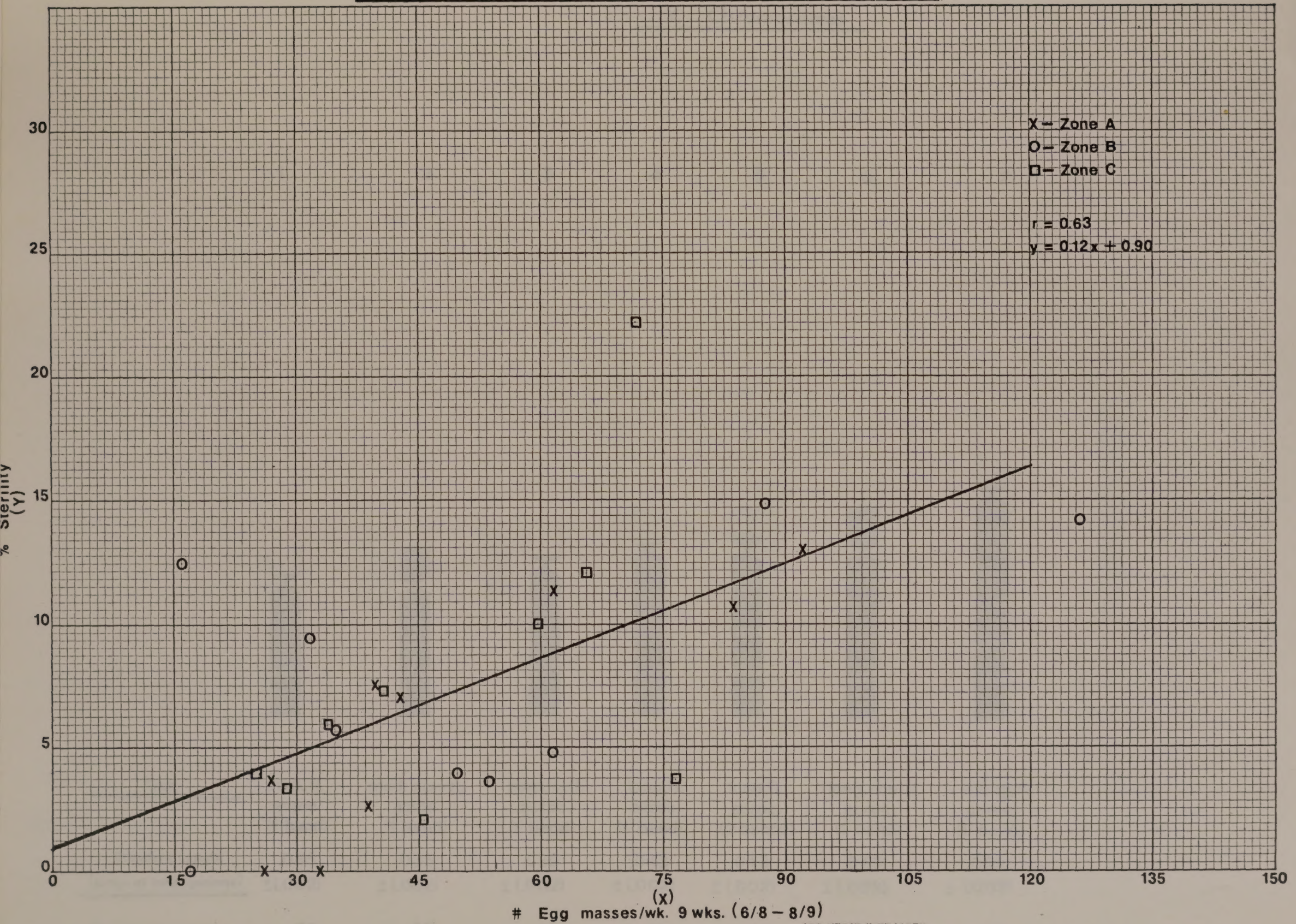
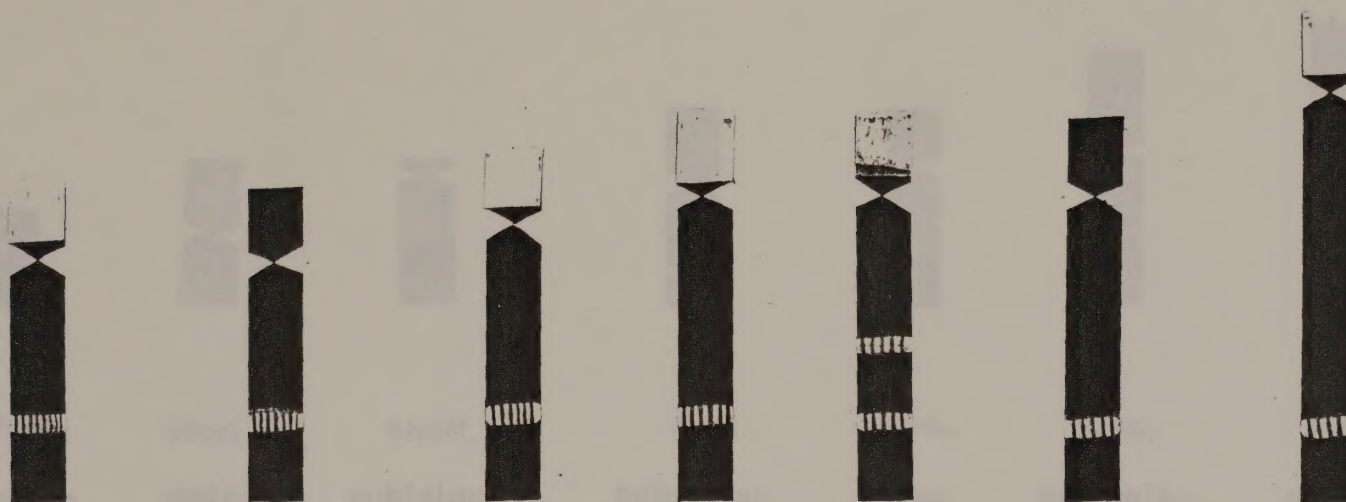


Figure 4.

ARRIAGA FIELD TEST

X Chromosomes

1 2 3 4 5 6 7



Type	: med., fl. acroc. (1 band)	med. non-fl. acroc. (1 band)	med.-l., fl. acroc. (1 band)	long, fl. acroc. (1 band)	long, fl. acroc. (2 bands)	long, non-fl. acroc. (1 band)	v. long, fl. acroc. (1 band)
Standard Length (prop. of total genome)	: .035 ± (.002)	.035 ± (.002)	.039 ± (.002)	.043 ± (.002)	.043 ± (.002)	.043 ± (.002)	.055 ± (.003)
Short arm/Long arm Ratio	: .30 ± (.05)	.30 ± (.05)	.27 ± (.05)	.25 ± (.05)	.25 ± (.05)	.25 ± (.05)	.20 ± (.05)

Figure 5.

ARRIAGA FIELD TEST

Y Chromosomes

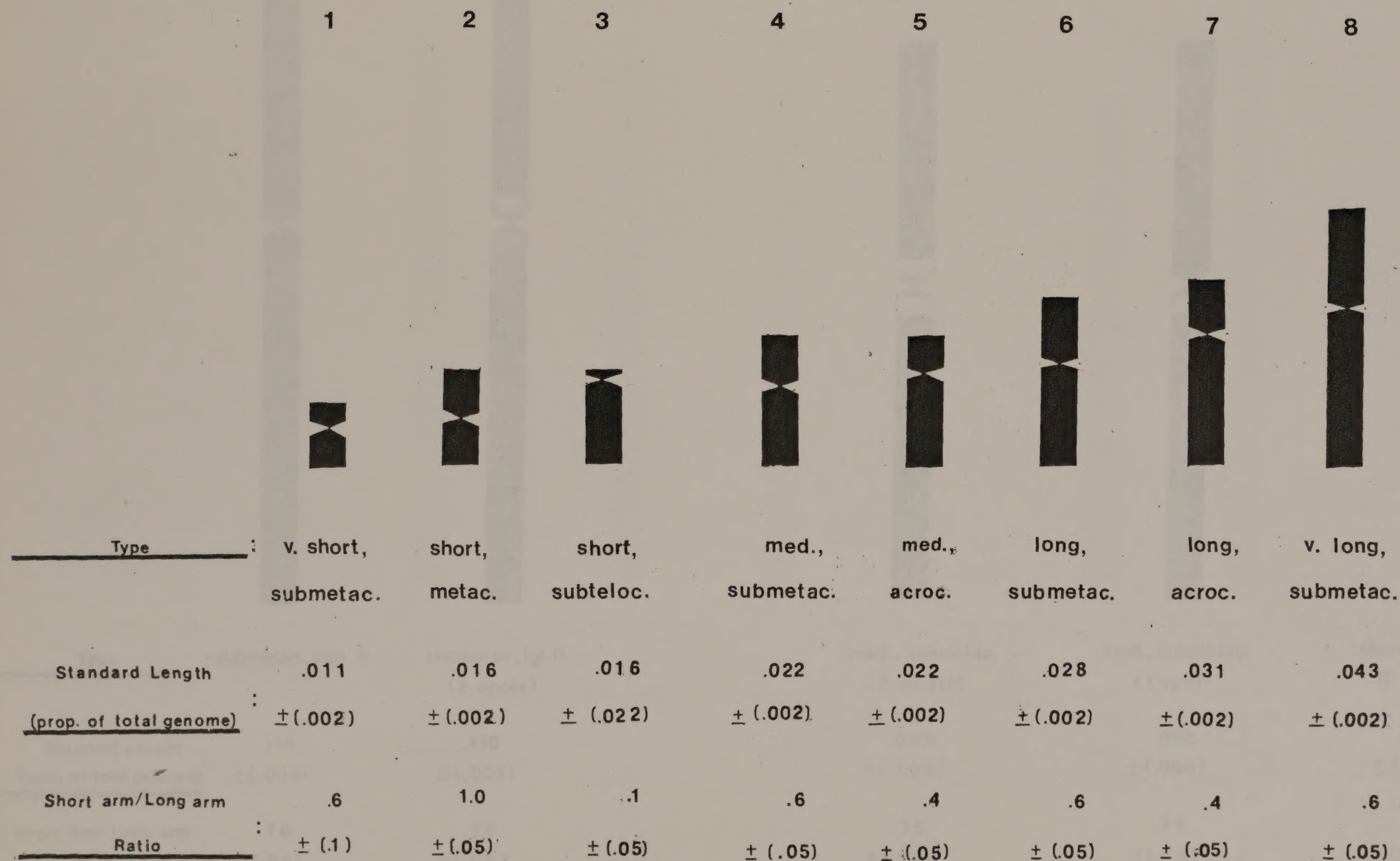


Figure 6.

ARRIAGA FIELD TEST

II Chromosomes

1

2



III Chromosomes

1

2

3



Type	: submetac., non fl.	submetac., lgt. fl. (2 spots)	med., submetac. (2 spots)	med., submetac. (1 spot)	short, submetac. (2 spots)
Standard Length (prop. of total genome)	.110 ± (.005)	.110 ± (.005)	.095 ± (.005)	.095 ± (.005)	.085 ± (.005)
Short arm/Long arm Ratio	.70 ± (.05)	.70 ± (.05)	.75 ± (.05)	.75 ± (.05)	.75 ± (.05)

Figure 7.

ARRIAGA FIELD TEST

IV Chromosomes

V Chromosomes

VI Chromosomes

1

2

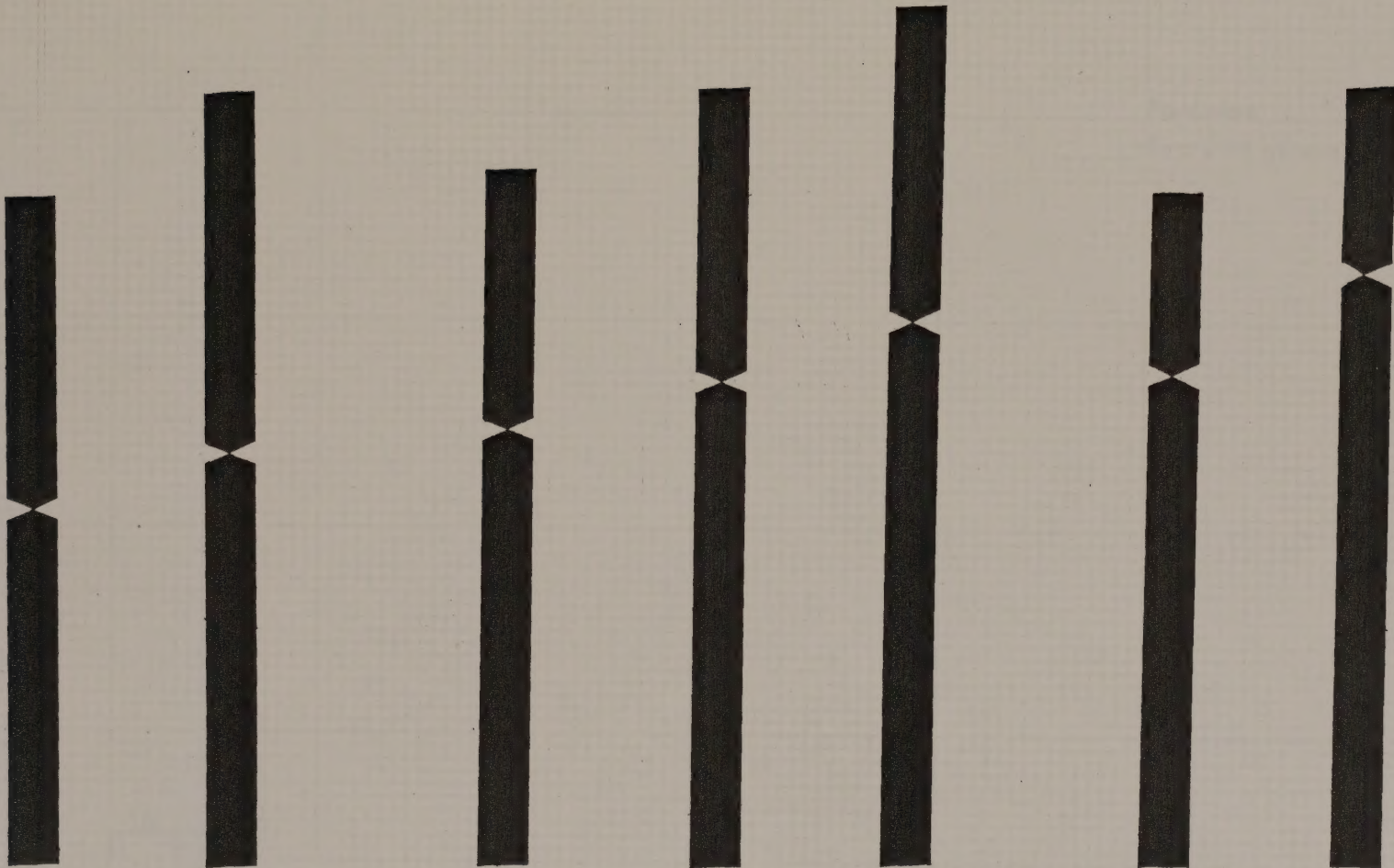
1

2

3

1

2



Type

short, metac.

long, metac.

short,
submetac.

medium,
submetac.

long,
submetac.

short,
acroc.

long,
acroc.

Standard Length

(prop. of total genome)

.082

.095

.085

.095

.105

.082

.095

± (.005)

± (.005)

± (.005)

± (.005)

± (.005)

± (.005)

± (.005)

Short arm/Long arm

Ratio

.85

.85

.60

.60

.60

.37

.32

± (.05)

± (.05)

± (.05)

± (.05)

± (.05)

± (.05)

± (.05)

Figure 8.

ARRIAGA FIELD TEST

Relative genetic distances between pairs of groups.

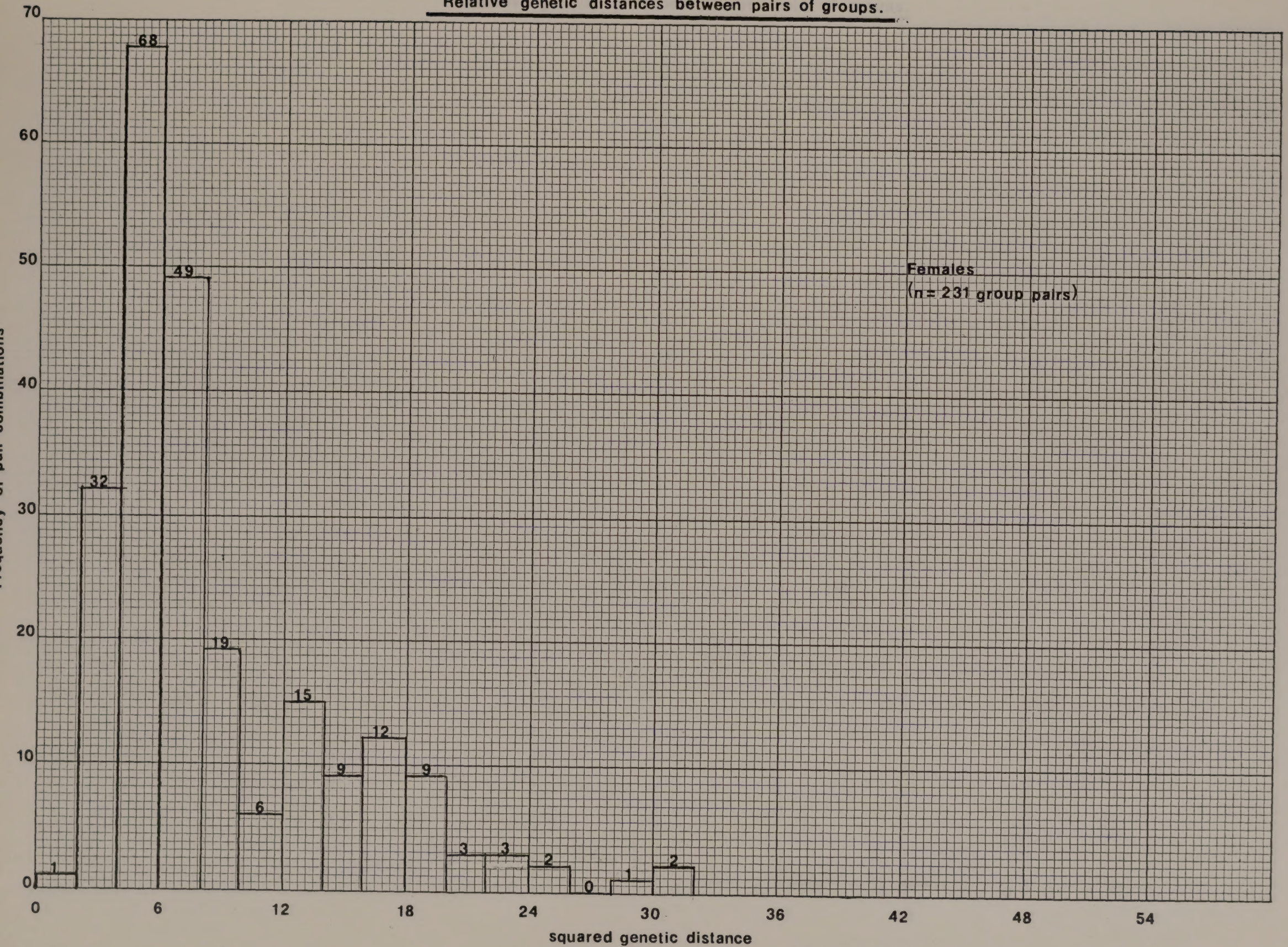
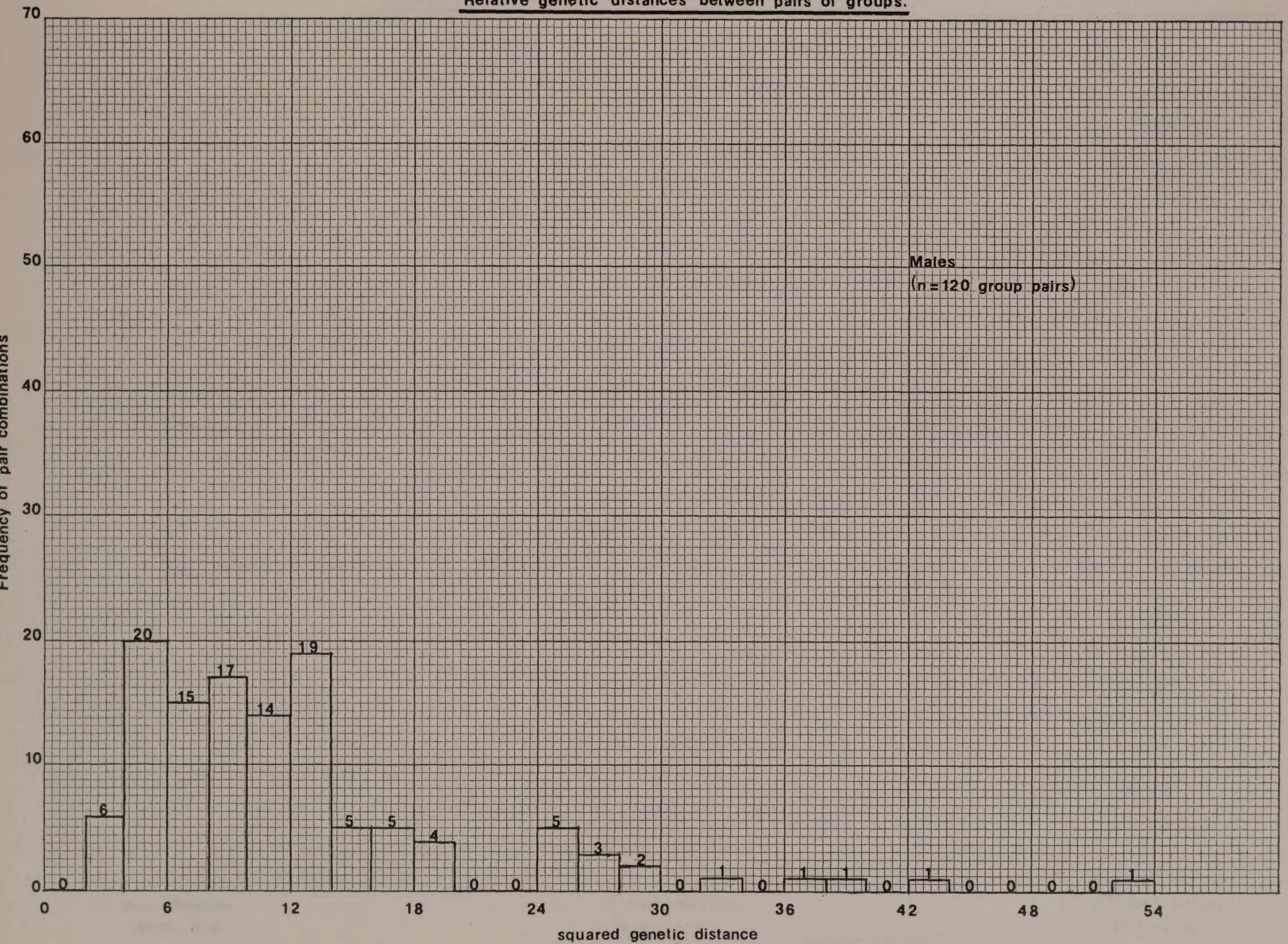


Figure 9.

ARRIAGA FIELD TEST

Relative genetic distances between pairs of groups.



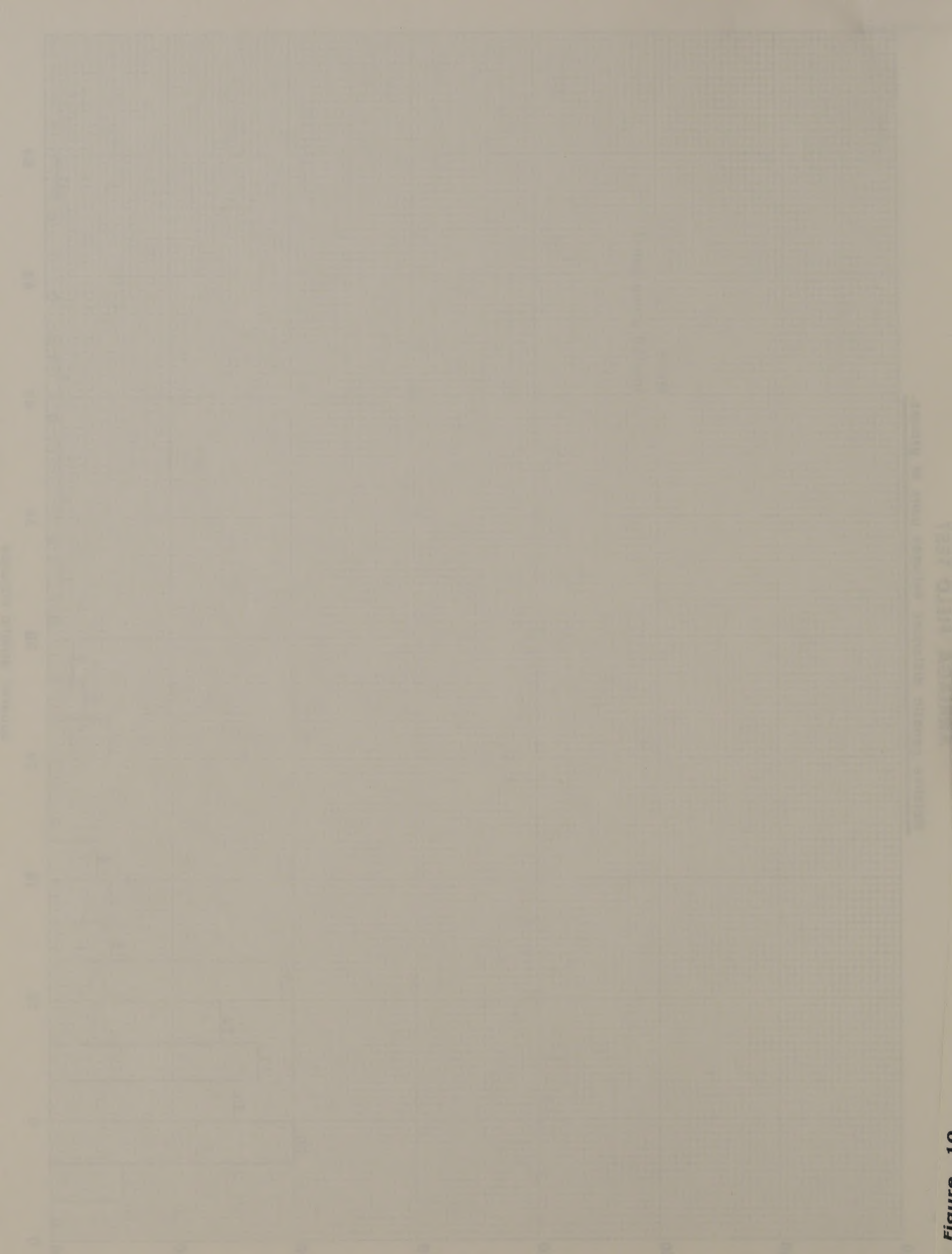


Figure 10

Figure 10.

ARRIAGA FIELD TEST

Frequency of the med., fl., acroc. X chromosome (X-1) over time.

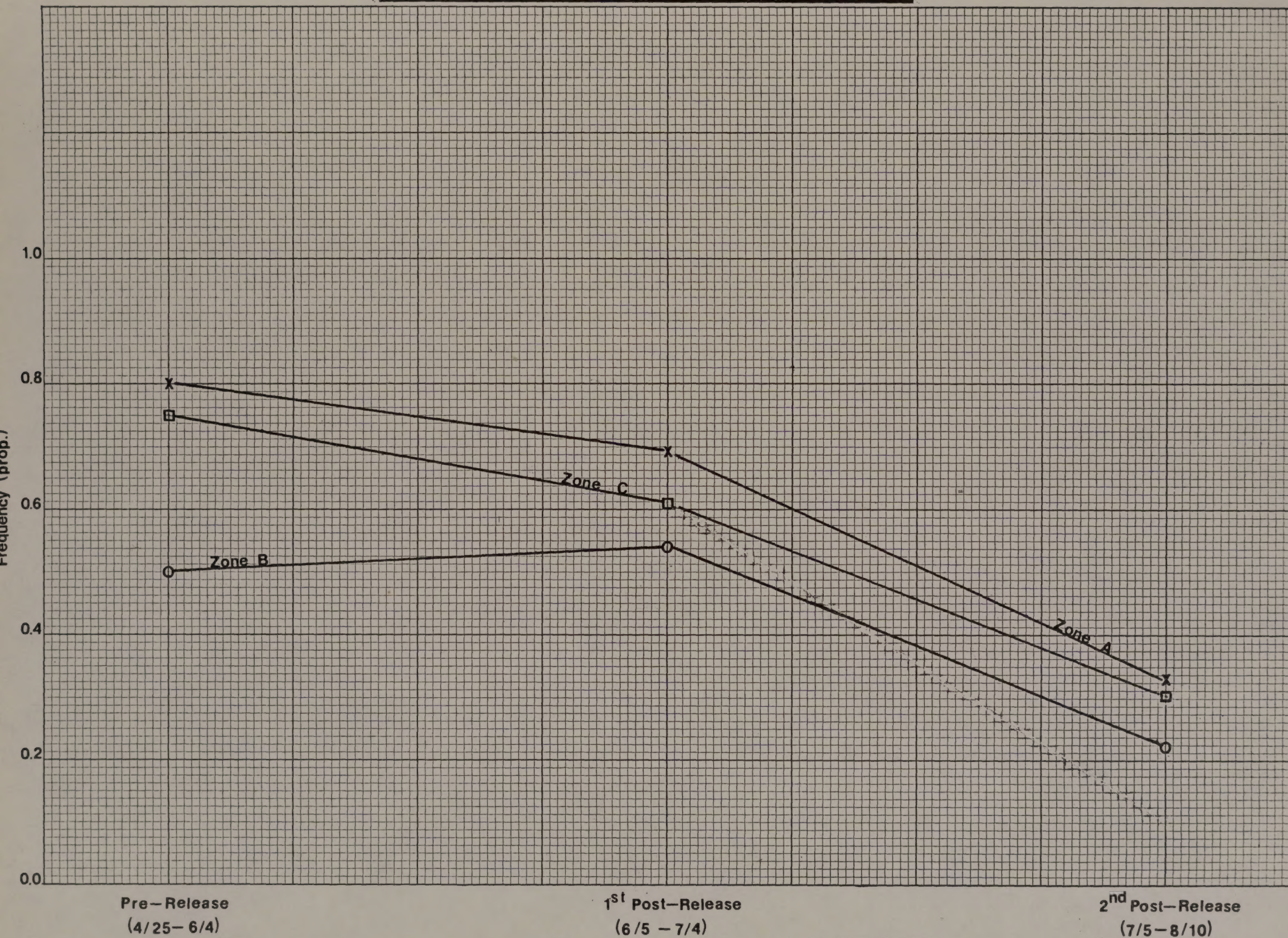
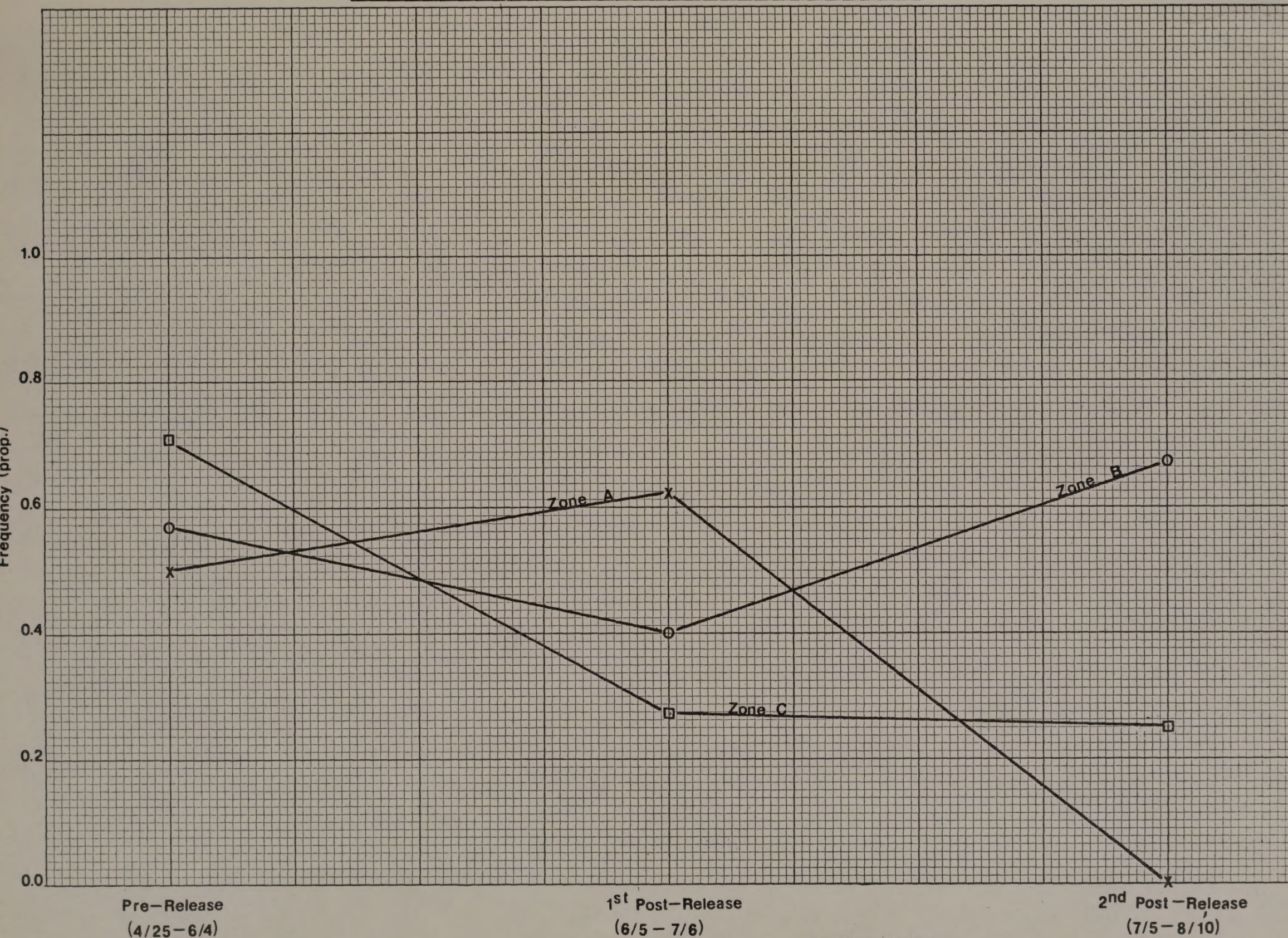


Figure 11.

ARRIAGA FIELD TEST

Frequency of the med., submetac. Y chromosome (Y-4) over time.



copies to

- UT
- Whitten
- Tuttle
- Bram
- O H G.